Water Content Determination of Rubber Stoppers Utilized for Sealing Lyophilized Pharmaceutical Products: Assessment of Two Karl Fischer Titration Methods

By

Laura Voth

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Chairperson John Stobaugh, Ph.D.

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Laird Forrest, Ph.D.

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Jim Searles, Ph.D.

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The Thesis Committee for Laura Voth
certifies that this is the approved version of the following thesis:

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Chairperson John Stobaugh, Ph.D.

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Abstract

In the pharmaceutical industry, the success of a new drug product is strongly impacted by the stability of the drug formulation. For many formulations, stability is governed by the drug product's water content, thus the ability to regulate this content determines the viability as a commercial product. Through lyophilization, the water content of a drug product may be controlled at the time of manufacture. However, over the product shelf life, additional water may be added or removed due to factors such as the storage environment and the drug's container/closure system, typically a vial, stopper, and cap. The water present in the rubber stopper may interact with the vial contents, potentially influencing the drug product's stability. Consequently, a formulation scientist must establish test methods capable of determining the initial and potentially subsequently changing moisture content for not only the lyophilized cake, but also the stopper.

Current literature describes two main analytical methods for measurement of the water content of rubber stoppers: a gravimetric method and a Karl Fischer (KF) titration method with oven. A third less common test method is the Karl Fischer titration method with tetrahydrofuran (THF) extraction. The results presented in this thesis thus describe the evaluation of the KF titration method utilizing an oven and the KF titration method utilizing a THF solvent extraction. The critical parameters of each test method were examined, and the advantages and disadvantages of these analytical methods were identified. Ultimately, it was concluded the drug product manufacturer must determine, based on knowledge of the drug product integrity and available manufacturing processes, the extent to

which the water content of the rubber stoppers must be quantified, as well as define the test method to be employed to perform this moisture content measurement.

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1 Chapter 1: Background/Introduction

1.1 Drug Stability

1.1.1 Lyophilization

During pharmaceutical drug product development, characterizing the stability of a given formulation and factors that affect stability of the active ingredient is critical to the long-term success of the drug product. For hydrolytically labile drug formulations, stability is frequently governed by the water content; therefore, control of this content determines if these formulations can become viable commercial products [1, 2]. In such circumstances, lyophilization (freeze-drying) is used for stabilization, as this process greatly reduces the drug product's water content at the time of manufacture [3, 4]. For other drugs in this category, once lyophilized, their glass transition temperature (Tg) is such that the product requires a low water content in order to retain the physical structure of the lyophilized cake [5, 6]. Unfortunately, lyophilization cannot guarantee a low water content over the entire shelf life of the drug product as additional factors subsequent to the freeze-drying process may influence the moisture content of the lyophilized formulation [7, 8]. One of these additional factors is the actual packaging of the drug product itself [9].

1.1.2 Packaging

Lyophilized products are commonly packaged within a glass vial with a rubber stopper. Although one purpose of the stopper is to serve as a barrier to prevent moisture from the external environment from entering the vial and lyophilized cake,

the stopper itself has a specific moisture content. This content is governed by the stopper formulation, moisture sorption properties, preparation prior to use, and storage [7, 10]. The water of the stopper is in constant flux with the moisture of the lyophilized cake until thermodynamic equilibrium is established between these components. Depending on the water concentration of the stopper and lyophilized product, moisture may either be released by the stopper and become associated with the drug product or be absorbed by the stopper from the formulation causing further desiccation of the lyophilized cake [11-13]. Research has shown that there is a correlation between moisture absorbed by the lyophilized product over a given time period and the moisture content of the stopper for that product [7, 10, 14]. Pikal and Shah recognized that it is the diffusion of water out of the stoppers, not the rate of absorption of the water by the product, that is the rate-limiting step in reaching an equilibrium between the stopper, product, and environment [12]. Additionally, studies have identified that the moisture vapor transmission rate of the stoppers, which is partially independent of the stopper moisture absorbance capabilities, also impacts the final water content of the lyophilized product [10, 15]. Consequently, the formulation scientist must establish analytical methods capable of characterizing the initial and potentially subsequently changing moisture content for not only the lyophilized cake, but also the stoppers, in order to delineate its contribution to the drug product stability.

1.2 Moisture in Rubber Stoppers

Internal and external components influence the water content of rubber stoppers, and it is the interplay between these components that provides the challenge for a researcher seeking to identify the proper stopper for a given product.

1.2.1 Internal – Composition/Matrix

Current rubber formulations utilized for the manufacture of pharmaceutical stoppers for lyophilized drugs commonly consist of several classes of ingredients: 1) elastomers, which are the base material, 2) curing agents, accelerators, and activators, which are important in the formation of the rubber cross-linkages, 3) antioxidants, which are stabilizers, 4) plasticizers, which are a processing aid, and 5) fillers and pigments that influence the physical properties. In addition, once manufactured, the stoppers may also be coated with silicone oil or B2 to act as a lubricant and aid in the machinability of the stoppers, or with a fluorinated polymer film, such as Teflon, PTFE, or ETFE, to serve as a barrier to minimize extractables from the elastomer and decrease absorption and adsorption [16]. As stopper formulations for lyophilized products should have low moisture vapor transmittance and low moisture absorbance, DeGrazio and Flynn explored common ingredients within these classes to identify which stopper components would exhibit the appropriate properties. They found that butyl and halobutyl elastomers are often used for the base material of rubber stoppers. In comparison with other elastomers, such as natural rubber, polyisoprene, and ethylene propylene diene monomer (EPDM), butyl and halobutyl elastomers have lower rates of moisture and oxygen transmission due to the presence of a greater number of methyl groups,

which make the elastomer non-polar. In addition, after polymerization, there are low levels of molecular unsaturation along the polymer backbone of butyl and halobutyl elastomers. DeGrazio and Flynn also determined that the amount of water-soluble impurities in these polymers, as well as the hydrophobicity of the fillers used in the stopper rubber formulations, impact the moisture absorbance of the stoppers. Water from the environment diffuses into the stopper matrix and pools around water-soluble impurities and hydrophilic fillers. In addition, impurities present in the curing agents and species formed during curing can also promote water absorbance [11]. Kruszynski et al. identified that a FluoroTec coating, promoted to minimize potential extractables, also serves as a moisture barrier and minimizes moisture uptake during sterilization [17]. Additional studies utilizing other coatings have had mixed results where some coated stoppers displayed a lower moisture content, while other coated stoppers showed no impact due to the coating, and finally others showed a higher stopper moisture content or a slower release of moisture even with oven drying [13, 16].

1.2.2 External – Sterilization and Storage

Beyond the physical components of the stoppers themselves, the processing and storage of these rubber stoppers also impact their moisture content. Numerous studies have been performed to investigate the water content of stoppers after steam sterilization, dry heat drying, lyophilization, and storage at multiple humidity conditions. The steam sterilization process has been found to add a high level of moisture to the rubber stoppers, with the amount of water absorbed being dependent on the stopper formulation [18]. Bromobutyl stoppers tend to absorb

less water during the autoclave sterilization compared to chlorobutyl stoppers [13]. To remove this added water, dry heat is often used to dehydrate the stoppers. Studies have shown that the amount of moisture removed is a function of the drying time as well as the stopper formulation [7, 17]. Depending on the formulation, some stoppers rapidly lose most of their water in the first one to two hours of drying, while other stoppers have a more even rate of water loss until the conclusion of the drying cycle [13].

The influence of the lyophilization process on the stoppers' moisture content varies based on their water content going into the process. Held and Landi saw a slight uptake in water during freeze-drying [18], while Donovan et al. saw no change in "dry" stoppers while "wet" stoppers had a loss in water. This "wet" stopper water loss was expected even without the lyophilization process due to the stoppers' moisture saturation and their known rapid moisture desorption [7]. Corveleyn et al. discovered no moisture loss by the stoppers during their lyophilization experiments [13].

Finally, after the drying and/or lyophilization process, stoppers may either gain or lose moisture depending on their storage conditions and formulation. Earle et al. found that 20-30% of the water lost during drying was regained after 13 days of routine storage in the sterile area of their filling facility [19]. Autoclaved stoppers that do not undergo a drying process and therefore tend to have a higher water content, most often will lose moisture over time to the environment while dried stoppers will tend to gain water from the environment [7]. Those stopper formulations that tend to have a higher water content, have a slower rate of

moisture saturation and therefore take longer to reach equilibrium with the environment. In addition, the higher the humidity during storage, the higher the equilibrium water content established in the stoppers [15].

1.3 Analytical Methods to Test for Stopper Moisture

Literature currently describes two main analytical methods by which the moisture content of a stopper is measured: a gravimetric method and a Karl Fischer titration method utilizing an oven.

1.3.1 Gravimetric Method

In the gravimetric method, which may also be called loss on drying, a stopper sample is weighed before and after a specified drying procedure at a particular temperature and drying length. The drying step removes the moisture from the sample and the change in weight indicates the initial moisture content of that stopper [19, 20]. One recognized drawback to this analytical method is its lack of specificity to water. Under particular conditions, volatile chemicals may also be released from the stopper sample and affect the measured results [21]. In addition, if the chosen test temperature is too high, the weight loss measured may be skewed by the decomposition of the stopper sample [22]. It is thereby difficult with some stopper formulations to identify the absolute water content of the stopper using the gravimetric method due to heat alteration of the sample [8].

1.3.2 Karl Fischer (KF) Titration Utilizing Oven

1.3.2.1 Karl Fischer Reaction

A Karl Fischer titration is fundamentally based on the Bunsen reaction between iodine and sulfur dioxide in an aqueous media.

$2H_20 + SO_2 + I_2 \rightarrow H_2SO_4 + 2HI$

Karl Fischer discovered that this reaction could be modified for the determination of water in non-aqueous solutions if the liberated acid is neutralized by an organic base and if an excess of sulfur dioxide is present. Fischer postulated the reaction as follows:

$2H_20 + SO_2(C_5H_5N)_2 + I_2 + 2C_5H_5N \rightarrow (C_5H_5N)_2H_2SO_4 + 2C_5H_5NHI$

A Karl Fischer reagent was therefore composed of a solution of iodine and sulfur dioxide in pyridine and methanol.

Since that initial discovery, the reaction has been closely studied and modified into two distinct steps as follows:

$ROH + SO_2 + R'N \rightarrow [R'NH]SO_3R + H_2O + I_2 + 2R'N \rightarrow 2[R'NH]I + [R'NH]SO_4R$

where ROH is an alcohol and R'N is a base.

The alcohol reacts with sulfur dioxide and a base to form an intermediate alkylsulfite salt, which is then oxidized by iodine to an alkylsulfate salt. This oxidation reaction also forms a hydroiodic acid salt and consumes water. This water consumption has a stoichiometric 1:1 relationship with iodine consumption. Presently, the typical reactive alcohol in Karl Fischer titrations is methanol. Pyridine formerly was the base, however due to safety issues, bases containing imidazole or primary amines are now being utilized instead [23-25].

1.3.2.2 Karl Fischer Titrations

There are two main titration types employing the Karl Fischer reaction that are utilized for water content testing at this time: volumetric and coulometric titration. For both titration types, the titer is based on iodine consumption due to the 1:1 relationship between water and iodine in the Karl Fischer reaction. As long as water is present in a solution containing Karl Fischer reagents, then the Karl Fischer reaction will occur and the iodine will be consumed. However, in the absence of water, iodine will remain present in the titration solution and an end-point is achieved [23]. Although the presence of iodine can be visually detected by a change in the color of the solution to a brown color [24, 26], automated detection is more precise. Most titration systems at this time thereby utilize a controlled current voltage detection system in which a constant current is applied to two platinum electrodes. The end-point of the titration is detected by a change in the voltage between the two electrodes. In the presence of high water content, a polarized voltage of 300-500 mV will be produced, but once all the water is consumed and therefore iodine is present in the solution, the voltage will suddenly drop to 10-50 mV. This drop in voltage will stop the automatic titration and the water content can be calculated [27].

In volumetric titration, a titrant containing iodine is utilized to titrate a standard of known water content. This allows for the determination of a water equivalency factor for the titrant, typically labeled in mg of water/mL of titrant. Next, the titrant is directly introduced to a Karl Fischer reagent solution containing the sample, and the volume of titrant needed to achieve the titration endpoint is identified. The

calculated water equivalency factor is then used to convert and calculate the final water content of the sample [26-30]. Because of its characteristics, volumetric Karl Fischer titration is most suited for samples with water content between 100 ppm to 100% water [23].

In coulometric titration, instead of having the iodine present in the titrant, the iodine is generated by a generator electrode via electrolytic oxidation of an iodide-containing solution which includes the sample [29, 31]. The amount of consumed iodine and thus the amount of consumed water is proportional to the total current utilized (current multiplied by time) during the titration to convert the iodide to iodine. The titration is completed when the detector electrode detects the presence of excess iodine in the solution (i.e. all the water is consumed) [27, 32, 33]. Unlike volumetric titration, where a water equivalency factor must be established, for coulometric titration, because electrons are serving as the titrant, one can measure the absolute quantities of water. Finally, because electricity can be measured in very tiny increments, coulometric titration is most often utilized for samples containing as low as 1 ppm up to 5% water [23].

For both volumetric and coulometric Karl Fischer titrations, there are some identified limiting factors that can significantly impact the use of these titration methods. First, the sample must typically be injected into the Karl Fischer solution. Solid samples must therefore be dissolved in anhydrous solvents that cannot interact with the Karl Fischer reagents or the electrodes, and the water content of these solvents must be determined [25, 30]. Solids that do not dissolve in organic solvents can reduce current efficiency by obstructing the electrodes and diaphragm

if present [27]. Second, some samples such as plastics and inorganic salts only release their water under very high temperatures [25]. Using the standard volumetric or coulometric Karl Fischer titration method, which is performed at room temperature, an accurate measurement of the water content of these substances therefore does not occur.

To address these limiting factors, a preparatory method using an oven has been developed. The oven serves as a sample preparation step that is performed in conjunction with a volumetric or coulometric titration. This method does not require sample dissolution into a liquid and allows for a range of test temperatures. Because of these characteristics, the Karl Fischer oven method is the second water content test method commonly found in the literature for rubber stoppers. Stopper samples are not easily dissolved in solvents that are compatible with the Karl Fischer titration method and also do not readily release their water at room temperature.

1.3.2.3 Karl Fischer Titration Utilizing Oven

For stopper moisture testing using the Karl Fischer titration method utilizing an oven, a stopper sample is weighed and then placed in a preheated oven. The heat of the oven vaporizes the sample's moisture and a dry gas carries the water from the sample oven to the Karl Fischer titration vessel where it is titrated until the ending criteria are met [7, 13, 21]. This titration may either be performed volumetrically or coulometrically depending on the expected amount of water present [27]. Unlike the gravimetric method, which also uses heat to extract the moisture, this technique measures only the water content of the stoppers because the Karl Fischer reaction is

specific to water. However, like the gravimetric method, this test method is dependent on the specific oven conditions, as increased temperatures extract greater amounts of bound water from the samples [34]. In addition, when testing at higher temperatures, one must ensure that the sample is not degrading to products that can interfere or skew the water content results [25].

1.3.3 Karl Fischer (KF) Titration Utilizing THF Extraction

Finally, a third analytical method for testing stopper moisture, not commonly found in the literature, is the Karl Fischer titration method using tetrahydrofuran (THF) as the solvent in the extraction step. In this test method, a stopper sample is soaked in the solvent THF, which due to its polar nature, extracts the water from the rubber stopper. Aliquots of the THF are then injected into the Karl Fischer titration vessel where they are titrated until the ending criteria are met [35]. Like the Karl Fischer titration method utilizing an oven, this titration method measures only the water content of the stoppers. However, unlike the other two analytical methods, temperature is not a standard parameter specified and investigated for this method. The THF extraction fluid is a volatile liquid with a boiling point of 66^{o}C [36] and thus the method is only performed at room temperature.

1.4 Objective

The main objective of the present investigation was thus to assess the capabilities of two Karl Fischer titration methods in determining the water content of rubber stoppers utilized for sealing lyophilized drug products. The study analyzed the Karl Fischer coulometric titration method with oven and the Karl Fischer coulometric

titration method with tetrahydrofuran (THF) extraction. Because the gravimetric method was not specific to water and was not a direct measurement, but instead a measurement of a change, this method was not reviewed in this research.

2 Chapter 2: Karl Fischer Titration Method Utilizing Oven

2.1 Background

Universal test parameters for determining the water content of rubber stoppers utilizing the Karl Fischer (KF) titration method with oven could not be found in the current literature. International Standard ISO:8362-5 outlines a composite test of small samples from the stopper flange of not less than ten stoppers. Samples are combined and tested at an oven temperature of 140°C and a suitable flow rate of dry nitrogen [37]. Donovan et al. chose a test temperature of 140°C in order to measure the free or unbound moisture in the stoppers. The stoppers were cut into four equal pieces and tested at a flow rate of 50-60 mL/min [7]. Wang et al. explored a range of oven test temperatures and found the measured water content of the stoppers to increase as the temperature increased. Their study concluded that only a portion of the water in the stoppers is releasable at any given oven temperature, as the water is bound to hydrophilic ingredients in the rubber material and is gradually released at higher temperatures. Thereby, the recommended KF oven temperature was 250°C based on accuracy and precision of their results when using a flow rate of 300 mL/min of dry nitrogen, and stoppers cut into not less than fifty pieces. It was noted however that it is only the releasable water that can impact the characteristics of a lyophilized product and in addition, the product will be exposed to only a portion of the stopper's surface. A test temperature of 250°C therefore is much higher than the standard storage temperature for a drug product and overestimates the releasable water that could interact with the drug [34]. Kruszynski et al. conversely utilized samples cut into 1/8 inch cubes and an oven

temperature of 200°C, as their data above this temperature was more variable and the samples began sticking together and charred at higher temperatures [17].

2.2 Materials

A Metrohm 860 KF Thermoprep Oven attached to a Metrohm 831 Karl Fischer Coulometer with diaphragm-less generator electrode and Hydranal Coulomat AG oven analytical solution was used for all water content determinations. Twenty millimeter West 4416/50 gray bromobutyl rubber single vent lyophilization stoppers were utilized in the analysis. In a nitrogen dry box, samples were cut and placed in 6-mL Karl Fischer oven vials, which were then hermetically sealed with aluminum caps with PTFE-backed silicone septa. Data was acquired using Metrohm Tiamo 2.3 software. Reference Appendices for specific instrument parameters such as start drift, voltage, minimum extraction time, and stop drift.

2.3 Test Method Assessment

2.3.1 Analytical Method

For each of the stopper moisture experiments performed with the Karl Fischer oven titration method, sample vials and caps were placed in a nitrogen dry box not less than 1 hour prior to sample preparation to minimize the moisture within the vial environment. Tubing scissors were used to cut samples of the appropriate size and shape from pre-weighed whole stoppers. Each sample was set in an oven vial, weighed, hermetically sealed into the vial, and then placed in a preheated oven with a specific temperature and carrier gas flow rate. All preparatory work was performed in the dry box with a relative humidity of less than 5% to reduce the

environmental influence on the measured water content of the stopper sample. Through the sample vial's septum cap, a specialized needle was inserted and the dry nitrogen carrier gas flowed into the vial. The carrier gas picked up the vaporized moisture released by the sample due to the oven temperature, and flowed out of the oven vial to the Karl Fischer coulometric titration cell. The sample's water content was then titrated until the specified ending criteria were met. For some of the experiments, blank controls (sealed vials without a stopper sample) were also performed in the same manner to identify the background water content from the environment (vial, seal, and atmosphere). However, for other experiments, due to the changing of variables such as temperature or carrier gas flow rate over the course of the experiment, the average blank control water content was set to zero micrograms (μg) of water. This was acceptable as these experiments were investigating the relationship between the changing variables and the water content, not the actual absolute water content. The amount of water in milligrams (mg) per stopper and the percentage of water per stopper were then calculated per the following equations:

$$mg_{Water}/Stopper = \frac{\left(W_t - \overline{W}_b\right)\left(W_E\right)}{\left(W_s\right)\left(1000\right)}$$

W_t	=	Instrument-generated weight of water titrated in the sample, in μg H_20
$\overline{W}_b$	=	Average weight of water titrated in the blanks, in μg H_20
W_E	=	Weight of entire stopper, in mg
W_S	=	Weight of stopper sample, in mg
1000	=	Conversion to mg

$$Percent_{water(sample)}(\%) = \frac{\left(\frac{W_S - \bar{W}_B}{W_A}\right)(100)}{(1000)}$$

W_S	=	Instrument-generated weight of water titrated in the sample, in μg H_20
$\bar{W}_B$	=	Average weight of water titrated in the blanks, in μg H_20
W_A	=	Weight of the sample, in mg
1000	=	Conversion to mg
100	=	Conversion to percent

2.3.1.1 Sample Type

A stopper used for lyophilized products consists of two main components, the leg(s) and the flange. The leg is the component that is inserted into the neck of the lyophilized product vial and is attached to the bottom side of the stopper flange. The leg may either be a single leg in a circular shape with a notch taken out called a single vent stopper or it may be two legs which are parallel to each other called a double vent stopper. The second component, the flange, is the top of the stopper which is circular in shape and overlaps the top of the vial neck to ensure a precise container seal between the vial and the stopper. It is through the flange that a needle is typically inserted to reconstitute the lyophilized product and withdraw the necessary drug dosage.

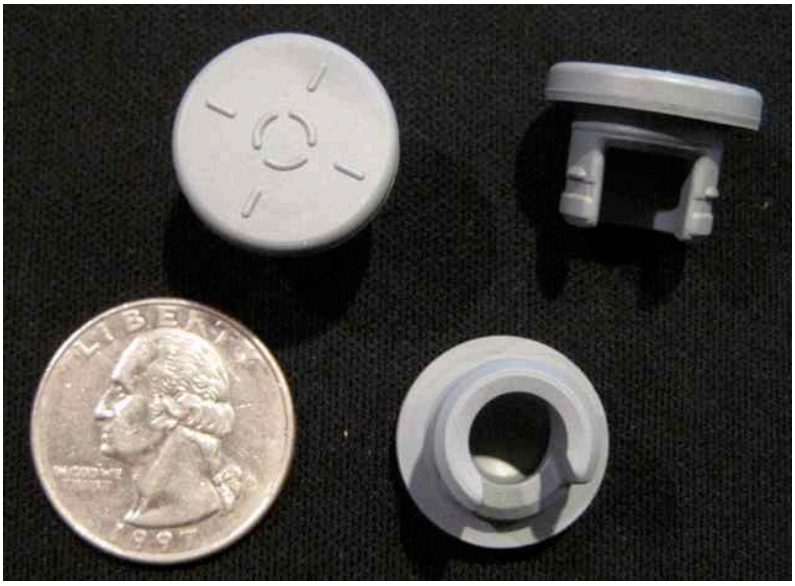

Image 2.1: West 4416/50 20-mm Single Vent Lyophilization Stoppers

Four trials were performed in which pieces of the flange, leg, or flange+leg (flange and leg connected) were cut from a stopper and tested for moisture content utilizing the standard oven method described. In the final experiment, flange and leg samples (a flange piece and a leg piece cut separately but placed together in the same sample vial for testing) were also analyzed. All tests were performed at an oven temperature of 200°C and a dry nitrogen carrier gas flow rate of 130 mL/minute.

2.3.1.2 Sample Size

Whole stoppers were cut into a range of sample masses and tested using the standard oven method. One trial consisted of testing samples cut from the stopper leg and was performed at an oven temperature of 220°C and a dry nitrogen carrier gas flow rate of 120 mL/minute. The second trial consisted of testing samples cut with the flange and leg connected (flange+leg) and was performed at an oven

temperature of 180°C and a dry nitrogen gas flow rate of 100 mL/minute. The final trial consisted of testing samples cut from the stopper flange and was performed at an oven temperature of 180°C and a dry nitrogen carrier gas flow rate of 100 mL/minute.

2.3.1.3 Oven Temperature

Flange+leg connected samples were cut from whole stoppers and tested over a range of oven temperatures using the standard oven method. Trial #1 utilized a dry nitrogen carrier gas flow rate of 150 mL/minute while the other three trials used a carrier gas flow rate of 100 mL/minute. Trials #1 and #2 had sample masses between 230-420 mg while trials #3 and #4 had sample masses between 100-200 mg.

2.3.1.4 Flow Rate

Flange+leg connected samples were cut from whole stoppers and tested over a range of dry nitrogen carrier gas flow rates using the standard oven method. Trial #1 utilized an oven temperature of 220°C while the other three trials used a temperature of 180°C. Trials #1 and #2 had large sample size ranges with masses between 155-460 mg, while Trials #3 and #4 had sample masses between 120-200 mg.

2.3.1.5 Sample Preparation

Sample Cutting

Whole stoppers were weighed and divided into their flange and leg sections using tubing scissors. Each of these sections was then cut into halves repeatedly until

consistently even halves were no longer possible utilizing the scissors. The smallest sample halves were then weighed.

Dry Box Exposure

Inside a dry box with a relative humidity of less than 1%, three approximately 155 mg flange+leg connected samples were cut from a whole stopper. Samples were weighed and then one sample was hermetically sealed in a sample vial and tested using the standard oven method while the other two samples were left in the dry box. Seven hours after being cut, one of the remaining samples was weighed, hermetically sealed into a sample vial, and tested. The final sample was weighed, hermetically sealed into a sample vial, and tested seventeen hours after being cut. All testing was performed at an oven temperature of 180°C and a dry nitrogen carrier gas flow rate of 75 mL/minute.

2.3.2 Test Method Assessment Results/Discussion

2.3.2.1 Sample Type

A comparison of the calculated water content of samples from different sections of a stopper reflected that the type of stopper sample did impact the calculated water content. As shown in Figure 2.1, flange samples achieved the lowest calculated water content, followed by leg samples, and then flange+leg connected samples. As the sample size range was within 50 mg for all types of samples for the final three trials, a difference in sample size did not appear to impact the results. Therefore, it is hypothesized that the flange component and the leg component of the stopper samples each released their water in a slightly different manner or at a slightly different rate. For future testing, a sample must thereby consist of both a section of

stopper flange and leg to achieve an accurate measurement of the total moisture content.

In addition, when comparing samples with similar masses consisting of flange and leg *connected* (flange+leg) versus *separate* flange and leg pieces tested together in the same sample vial (flange and leg), the average results were 14.06 mg water/stopper +/- 0.29 mg and 14.04 mg water/stopper +/- 0.19 mg respectively. These results were subjected to a t-test and found equivalent with no statistical difference at a confidence level of 99% ($t_{calculated}=0.91 < t_{table}=4.60$). This fact made it simpler in future test method design to achieve a more consistent sample shape and size from sample to sample and test to test. When the flange and leg were connected, the shape of the stopper as well as its flexibility made it awkward and difficult to consistently cut. However, with the flange disconnected from the leg, both pieces could more evenly be cut into the appropriate size and shape.

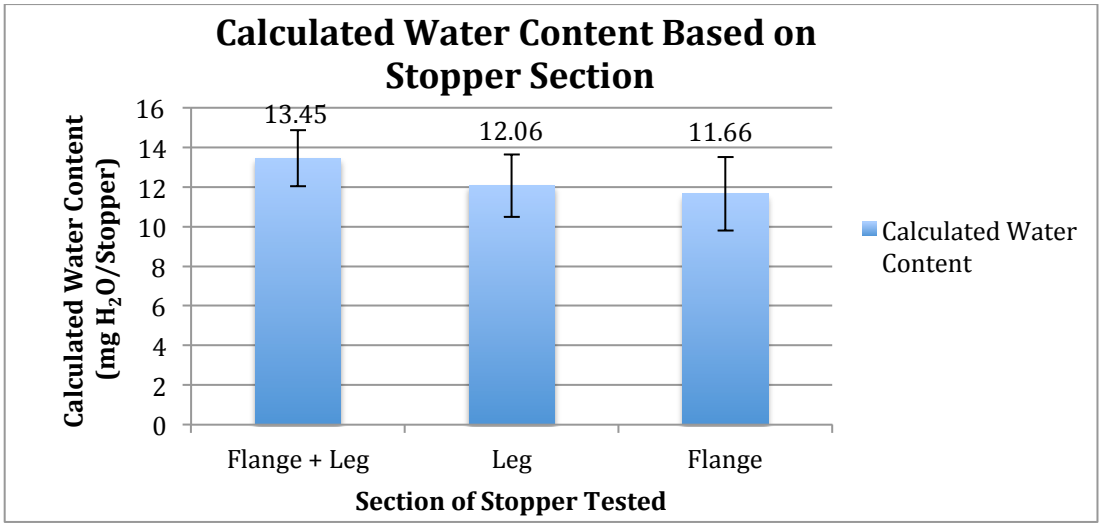

Figure 2.1: Calculated Water Content Based on Stopper Section

2.3.2.2 Sample Size

The sample size trials uncovered two key factors in the testing of the water content of rubber stoppers using the Karl Fischer coulometric titration method with an oven. First, as reflected in Figure 2.2, the type of sample and more precisely the sample shape used during testing impacted the measured and thereby calculated water content. (Note, the leg sample trial was performed at a higher temperature and flow rate than the other two trials). This result mimicked the data obtained via the sample type trials. Second, the sample size indirectly influenced the measured and calculated water content of the sample, as long as the sample consisted of both the flange and leg or just the flange, because of the impact of sample shape. As displayed in Figure 2.2, if a sample consisted of only a piece of the stopper leg, then there was no correlation between sample size and measured water content up to a mass of 330 mg. However, if a sample consisted of a piece of the flange+leg of a stopper connected or a piece of the flange, then as the sample mass increased, the sample shape changed, and thus the measured water content decreased.

Upon examining the stopper samples tested, it became apparent that the length of diffusion for the stopper's water from within the sample to the surface for evaporation and movement to the titration vessel was an important variable during this testing. In experiments performed by Wang et al., it had been determined that sample thickness and the number of sample pieces into which a stopper has been cut, impacted the percent moisture level calculated for a given stopper. They concluded that large sample sizes can lead to diffusion barriers during testing and thereby lower moisture content results [34]. Therefore, for the leg samples tested

in the sample size trials, although the width of the samples increased as the sample mass increased, the thickness of the leg remained the same. This thickness was the shortest path for the water diffusion in the leg samples, so the increased sample mass did not impact the resulting water content measured, as the water continued to diffuse out via the shortest path or the sample thickness. However, for the flange+leg connected samples or flange only samples, as the sample masses increased, the water diffusion path length changed. Based on results of the sample type trials, these samples were cut in a wedge shape to ensure a consistent sample of all appropriate areas of the stopper (interior and exterior sections of flange and leg). With this wedge shape, as the sample size increased, the diffusion from the leg section of the samples was not altered, just like with the solely leg samples' diffusion, but the diffusion from the flange section of the samples changed. At smaller masses, the flange section was thinner and less pie-shaped and therefore had a more even width from tip to exterior edge. The shortest diffusion path length was therefore via the width of the sample. However as the sample size increased, the width increased, and the flange section became more pie shaped. The shortest diffusion length thereby varied from the tip where it was the width of the sample to the exterior edge where it was the height of the flange. With a longer diffusion path length, it took longer for the water within the interior of the stopper sample to reach the surface and thereby be measured. Conversely, the software for the Karl Fischer titration method with oven was programmed to discontinue the titration when the rate of change in water content within the titration cell was below a certain level. Thereby if the sample mass was too large (i.e. the sample shape a thicker wedge)

and thus the diffusion path length of the stopper sample too long, then the water titration was stopped by the instrument before all the water from the stopper had diffused out of the sample and been carried to the titration cell for analysis. Although the specified rate at which the instrument discontinued the titration could have been changed, it was predicted if a lower rate was chosen, then the test time would increase and the background drift within the titration cell would become an issue. Therefore, using the current titration parameters, based on the data obtained, a sample size of not more than 75-100 mg was proposed to ensure that the flange had a more uniform width from tip to exterior edge and thereby the sample shape, as reflected through the sample size, and diffusion path length did not largely impact the calculated stopper water content.

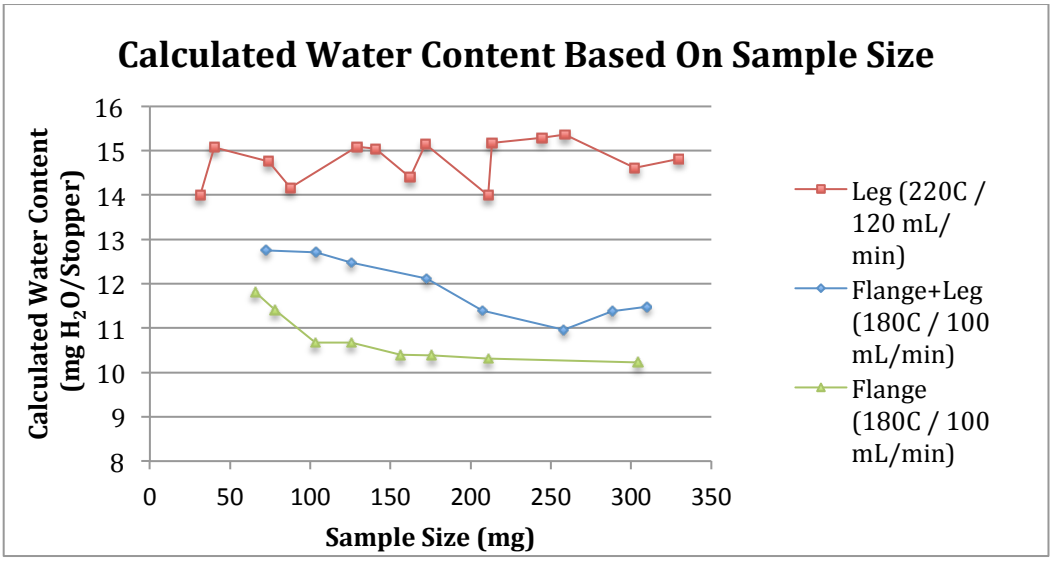

Figure 2.2: Calculated Water Content Based on Sample Size

As displayed in Figure 2.3, the increase in titration test time with an increased mass was greater (i.e. a steeper slope) for the flange and flange+leg connected samples, compared to the leg samples' test time increase. This also reinforced the fact that

the diffusion of water within the sample was the rate-limiting step during the titration. For the leg samples, since the shortest diffusion path length remained the thickness of the sample throughout the range of sample masses tested, the test times minimally increased due to the fact that the sample masses were getting larger and therefore had more water. With the increased sample size for the flange and flange+leg connected samples, the sample shape because a thicker wedge and the diffusion path length thus became longer, therefore there was a larger increase in test time. It took a greater amount of time for the titration to be completed not only because of the greater amount of water with the increase in sample masses, but also because it took longer for the water to reach the samples' surface to be released due to the change in the sample shape with the increased mass.

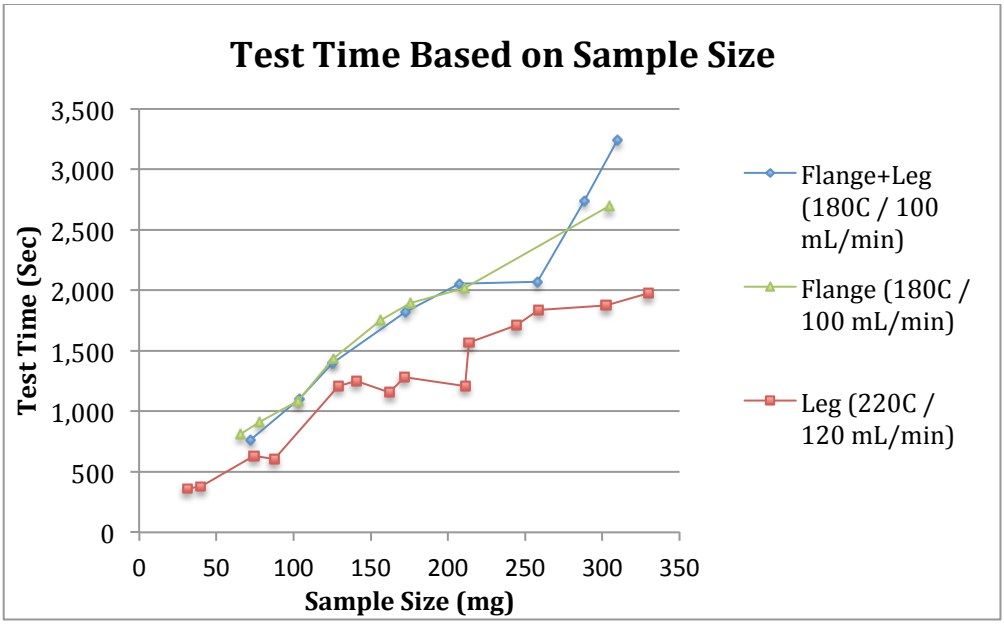

Figure 2.3: Test Time Based on Sample Size

2.3.2.3 Oven Temperature

As shown in Figure 2.4, in all four trials, as the oven test temperature increased, the calculated water content per stopper increased. Similar to the data collected during the sample size trials, Trials #1 and #2 with the higher sample masses, had lower calculated water contents. In addition, because their sample sizes included a broader range, the results were more variable and thereby more difficult to analyze. Therefore, looking at Figure 2.5, which consists solely of data from Trials #3 and #4, since their sample masses were lower and in a smaller range, a better analysis occurred. At the lowest oven temperatures below the boiling point of water (100°C), a water content of less than 2 mg/stopper was calculated based on the measured water content for the samples. Between 100°C-120°C, there was an increase in water measured representing the surface water of the stopper sample. From 120°C through 180-200°C, there was a near linear increase in water content as an increased amount of water from the interior of the stopper diffused out with the increase in temperature. Finally beyond 200°C, there was another change to the measured water content from the stopper samples. Upon examining the physical properties of the tested samples, it was found that around 200-210°C, the stopper samples became softer and sticky and began to swell and eventually crack. At the highest temperatures, the samples deformed and took the shape of the sample vials. It was therefore believed that at temperatures above 200°C, the rubber composition began to degrade, resulting in an increase in the release of water.

For future testing, an oven test temperature of 180°C was therefore proposed as this temperature provided the most complete measurement of the total water content of

the stopper. This temperature was along the linear range of the oven temperature versus water content comparison thereby representing a near direct correlation between the two parameters. In addition, because it was on the higher end of the linear range and because higher temperatures evolved greater amounts of water from the samples, the chosen temperature of 180°C provided a more accurate measurement of the absolute water content of the stopper. This oven temperature was high enough to measure more than just the surface water of the sample, but low enough that it was not skewed by degradation of the rubber.

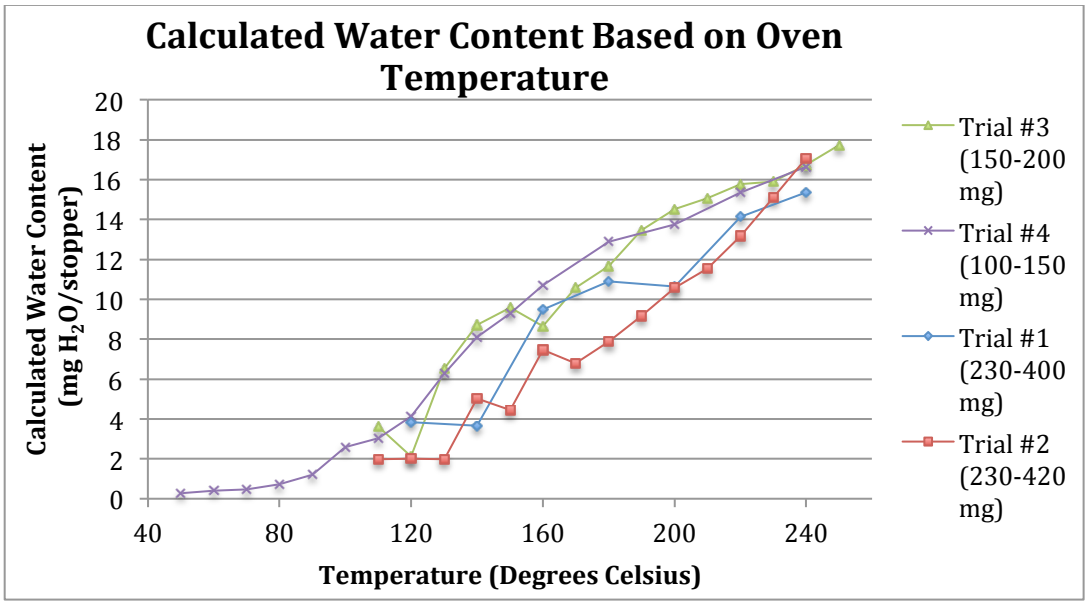

Figure 2.4: Calculated Water Content Based on Oven Temperature

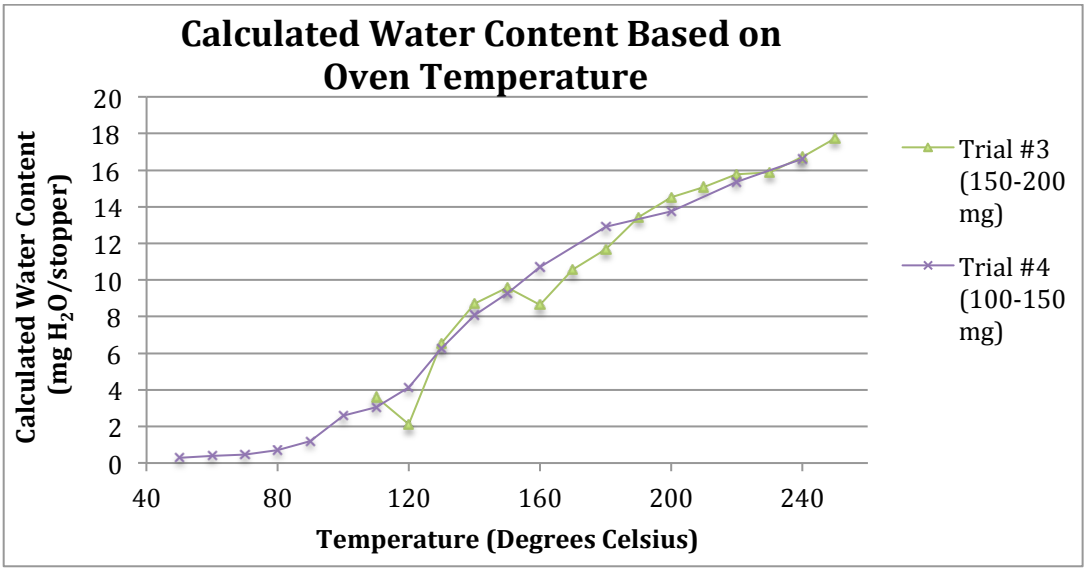

Figure 2.5: Calculated Water Content Based on Oven Temperature-Trials #3 and #4

2.3.2.4 Flow Rate

As shown in Figure 2.6, within the range of 10 mL/minute to 150 mL/minute, the flow rate of the dry nitrogen carrier gas did not have a large influence on the measured and calculated water content of the stopper samples. As expected, since Trial #1 was performed at a higher oven temperature of 220 °C versus 180 °C for the other three trials, a higher water content was calculated per stopper during this trial. Additionally, since Trial #2 had larger sample masses and a broader range of masses, it had more variable results with lower calculated water contents. The tested carrier gas flow rates versus the test times of Trials #3 and #4, which had similar sample masses tested, are compared in Figure 2.7. It was found that flow rates above 45 mL/minute had a consistent test time up to a flow rate of 150 mL/minute. This aligned with the results of previous trials that suggested that it was the diffusion path length from the interior of the stopper to the surface, not the

carrier gas flow rate that was the rate-limiting step in the water content measurements. Based on this figure, below 45 mL/minute, the carrier gas flow rate was the rate-limiting factor in the water content titration as it took longer for the water to be carried to the titration cell and therefore the analysis time was longer. However, above 45 mL/minute, the diffusion path length for the water within the stopper sample became rate-limiting for the titration and so an increased carrier gas flow rate did not decrease the test time.

Finally, an additional factor recognized when considering the carrier gas flow rate was the diffusion of the water from the carrier gas to the solution in the titration vessel. The slower the flow rate, the less mixing that occurred in the titration vessel and therefore the thicker the boundary layer through which the water had to diffuse from the carrier gas. Thus, at lower flow rates not only did the water reach the titration cell at a slower rate, but it also took longer for the water to diffuse into the titration solution, thereby increasing the titration time. Therefore as a whole, the test time was not just a reflection of the carrier gas flow rate. The test time was influenced by the diffusion rate and path length of the water from the carrier gas into the titration cell solution as well as the diffusion rate and path length of the water from within the stopper to the carrier gas.

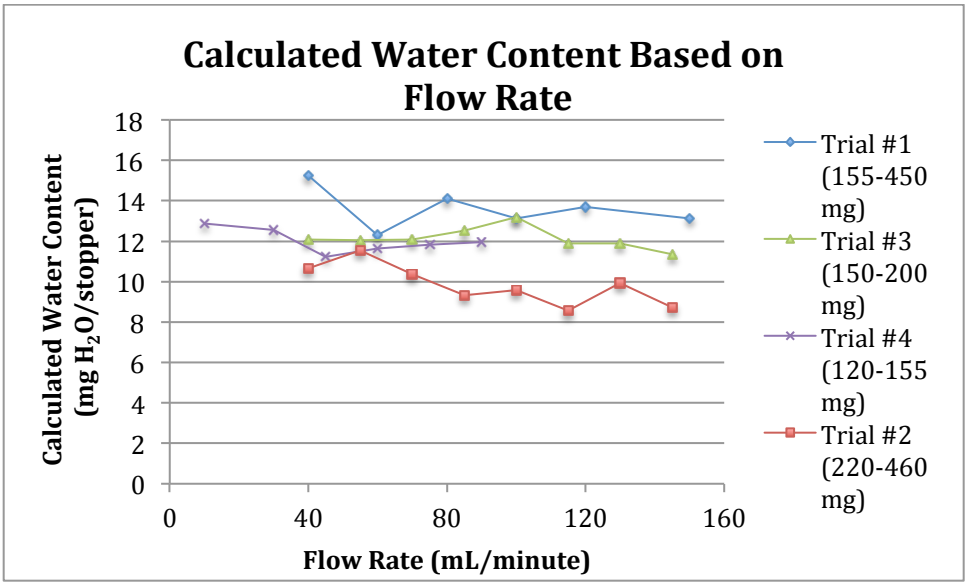

Figure 2.6: Calculated Water Content Based on Flow Rate

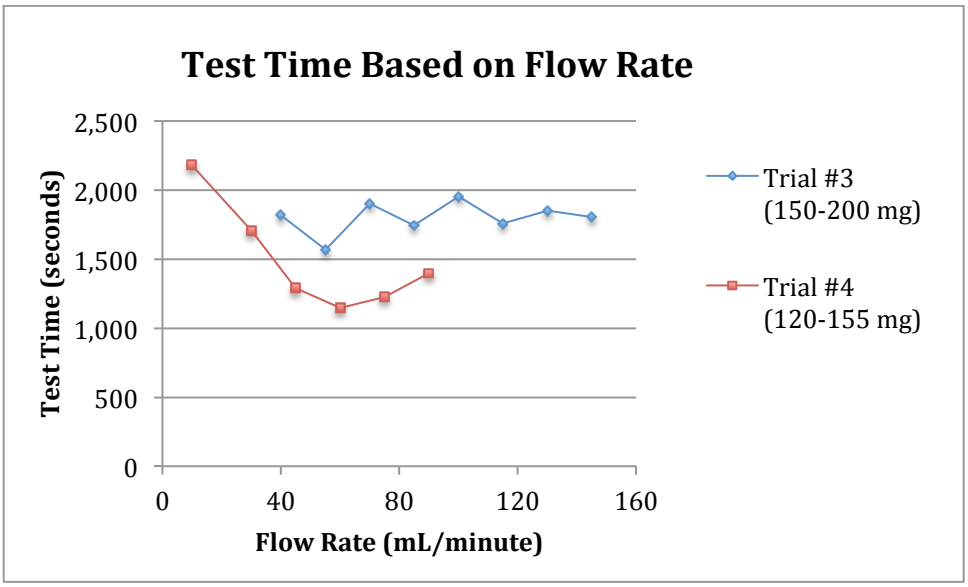

Figure 2.7: Test Time Based on Flow Rate-Trials #3 and #4

2.3.2.5 Sample Preparation

Stopper Cutting

Reflected in Image 2.2, the smallest flange piece that could consistently be cut was a pie-shaped sliver comprised of one-sixteenth of the total flange. The smallest leg

piece that could consistently be cut was one-eighth of the total leg. For this specific West 4416/50 gray bromobutyl rubber single vent 20-mm stopper type, each of these pieces was approximately 100 mg. However as shown in Table 2.1, the measured sample masses had a large standard deviation and percent RSD for each type of sample. Due to the flexibility of the rubber stoppers, consistently cutting samples into halves using scissors, without the rubber bending and stretching with the cut, was extremely difficult. Although other cutting methods, such as using a utility knife or serrated knife, were attempted at initiation of the oven trials, a better cutting method was not identified.

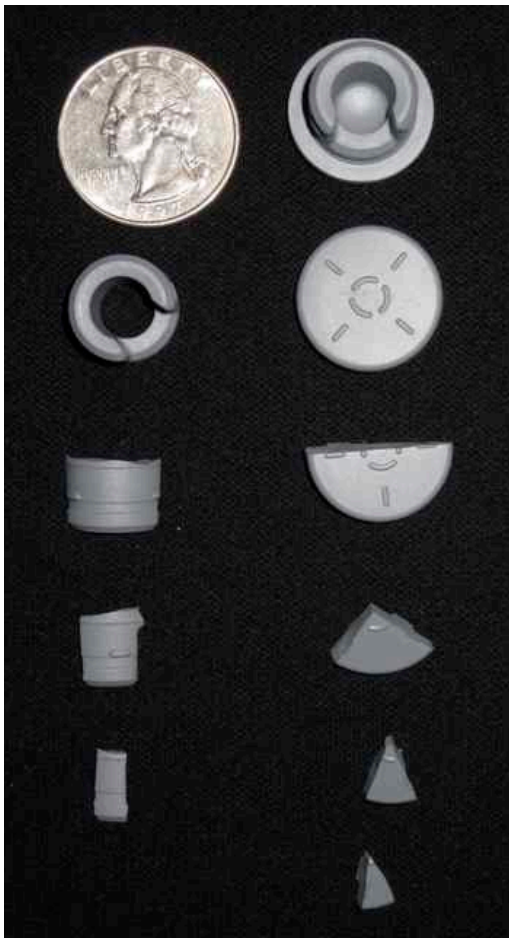

Image 2.2: Stopper Flange and Leg Cut Samples

Table 2.1: Stopper Cutting Sample Weights

Stopper Sample Weights (mg)									
	Whole Stopper	Leg Sample 1	Leg Sample 2	Leg Sample 3	Leg Sample 4	Flange Sample 1	Flange Sample 2	Flange Sample 3	Flange Sample 4
Stopper #1	2406.38	112.14	104.78	114.57	101.90	78.12	88.90	93.88	86.17
Stopper #2	2417.62	115.94	105.18	93.33	76.63	89.30	95.79	72.35	138.53
Stopper #3	2418.43	89.71	135.24	133.88	92.36	104.14	98.47	84.62	92.27
		Leg Average Weight		106.31		Flange Average Weight		93.55	
		Standard Deviation		15.75		Standard Deviation		16.59	
		%RSD		14.81%		%RSD		17.74%	

Dry Box Exposure

The length of time a cut stopper sample was exposed in a dry box with a relative humidity of less than 1% prior to being hermetically sealed in a sample vial impacted the measured water content of the sample. As documented in Table 2.2, based on the stopper's percent water calculated at time zero and the time zero sample weights, the 7 hour sample should have had a water content of 784.01 µg and the 17 hour sample should have had a water content of 869.41 µg. Instead the measured water was 741.69 and 751.75 µg respectively, which reflected a decrease in the water content prior to sealing and measuring the moisture at the specified time intervals. Although there was little recorded change in milligrams (mg) in the sample weights after 7 and 17 hours of exposure in the dry box, this was simply due to the fact that the balance was only calibrated down to 20.00 mg +/- 0.02 mg and thus was not sensitive enough to reflect the smaller change in micrograms (µg). Due to the very low humidity of the dry box (less than 1%), as the samples sat in that atmosphere, they lost water to the environment. This process would have continued until an equilibrium was reached between the water content

of the stopper samples and the dry box atmosphere. Thus, a dry box was and should be used during sample preparation to prevent an increase in the sample's water content due to the environment in which it was and will be prepared. However, based on this experiment, the length of exposure within the dry box must also be controlled to prevent a loss of water to that environment as well.

Table 2.2: Titrated Water Based on Dry Box Exposure

Titrated Water Based on Dry Box Exposure										
Sample	T:0 Weight (mg)	Final Weight (mg)	Difference (mg)	Calculated Water Content (mg H2O/ stopper)	% Water	Theoretical Water based on T:0 (µg)	Actual Titrated Water (µg)	Actual Titrated Water-Blank (µg)	Difference (µg)	Difference (mg)
T:0	164.41	NA	NA	12.73	0.53	870.46	876.52	870.46	NA	NA
T:7 Hr.	148.08	148.10	-0.02	12.04	0.50	784.01	747.75	741.69	42.32	0.04
T:17 Hr.	164.21	164.20	0.01	11.01	0.46	869.41	757.81	751.75	117.66	0.12

Average Blank (µg)= 6.06 Theoretical Water Based on T:0 = T:0 Weight * % Water at T:0 (0.53%) * 1000

2.4 Final Test Method

2.4.1 Analytical Method

Based on the results from the test method assessment experiments, the finalized analytical method for the Karl Fischer titration method with oven is as follows. Sample vials and caps are placed inside a dry box with a relative humidity below 5%, for not less than one hour prior to sample preparation to ensure a low water content within the vial environment at time of testing. A 20-mm stopper is weighed and cut with tubing scissors to obtain a representative sample for testing. For simplicity of cutting and consistency of sample shapes and masses, the stopper is cut into its two components, the flange and the leg sections. Then, the flange is cut into sixteenths to achieve thin, slightly pie-shaped pieces and the leg is cut into eighths to achieve thin rectangular cubes. One-sixteenth of the flange and one-eighth of the

leg are placed together into a tared 6-mL KF oven vial for a total sample mass of approximately 200 mg. By testing samples from both components of the stopper, one addresses the bias reflected in the sample type experiments. In addition, by utilizing a piece of the flange and a piece of the leg separate but in the same vial, instead of the two components connected as a single sample, the sample masses are both around 100 mg and therefore their separate masses and shapes and the resulting diffusion path lengths should not largely impact the water content measured. The actual sample mass is weighed, and the vial is immediately hermetically sealed to prevent water loss due to the anhydrous nature of the dry box. Three additional 6-mL KF oven vials are prepared in the same fashion without the presence of a stopper to serve as the blank controls. These multiple blanks help to account for vial-to-vial atmospheric moisture variance that is not a reflection of the water content of the stopper samples themselves. All preparatory work is performed in a dry box with a relative humidity of less than 5% to reduce the environmental influence on the measured water content of the stopper samples. The blank or sample vial is then set in the oven at a temperature of 180^{o}C for sample testing as this temperature reflects the most accurate total moisture content for the stopper. A nitrogen carrier gas flow rate of 100 mL/minute is used as it is in the center of the range (45 mL/minute to 150 mL/minute) that achieves approximately the same results and has nearly the same test time during the experiment trials. The sample's water content is then titrated using the Karl Fischer coulometric titration method with oven until the specified ending criteria are met. The amount of water

in milligrams (mg) per stopper and the percentage of water per stopper are then calculated per the following equations:

$$mg_{Water}/Stopper = \frac{\left(W_t - \overline{W}_b\right)\left(W_E\right)}{\left(W_s\right)\left(1000\right)}$$

W_t	=	Instrumented-generated weight of water titrated in the sample, in μg H_20
$\overline{W}_b$	=	Average weight of water titrated in the blanks, in μg H_20
W_E	=	Weight of entire stopper, in mg
W_S	=	Weight of stopper sample, in mg
1000	=	Conversion to mg

$$Percent_{water(sample)}(\%) = \frac{\left(\frac{W_S - \bar{W}_B}{W_A}\right)(100)}{(1000)}$$

W_S	=	Instrument-generated weight of water titrated in the sample, in μg H_20
$\overline{W}_B$	=	Average weight of water titrated in the blanks, in μg H_20
W_A	=	Weight of the sample, in mg
1000	=	Conversion to mg
100	=	Conversion to percent

2.4.2 Final Test Method Results/Discussion

As reflected in Figure 2.8, using the final Karl Fischer titration method with oven, the calculated water content results for the three stoppers tested were very consistent with a mean of 12.5 mg water/stopper +/- 0.3 mg. The separation of the flange from the leg allowed for easier manipulation of the sample for cutting. However, the flexibility of the rubber still made it difficult to achieve consistent sample sizes as the samples became smaller. As expected, the sample weights for

each piece of flange and leg were approximately 100 mg and the total sample weight tested for each stopper was approximately 200 mg (196 mg, 195 mg and 202 mg respectively). This led to sample test times of 33-34 minutes, which aligned with previous testing times for this sample weight. Physical observation of the samples after testing found that they were not deformed or sticky and therefore the test temperature utilized was below the point of degradation for this type of stopper. Finally, because the standard deviation between the three stoppers' results was low, this implied that the sample sizes and shapes were sufficiently small and thin so that the diffusion path length for the water within the stopper was not greatly different from sample to sample which alleviated the variable results and test times that occurred when the water diffusion path lengths varied greatly.

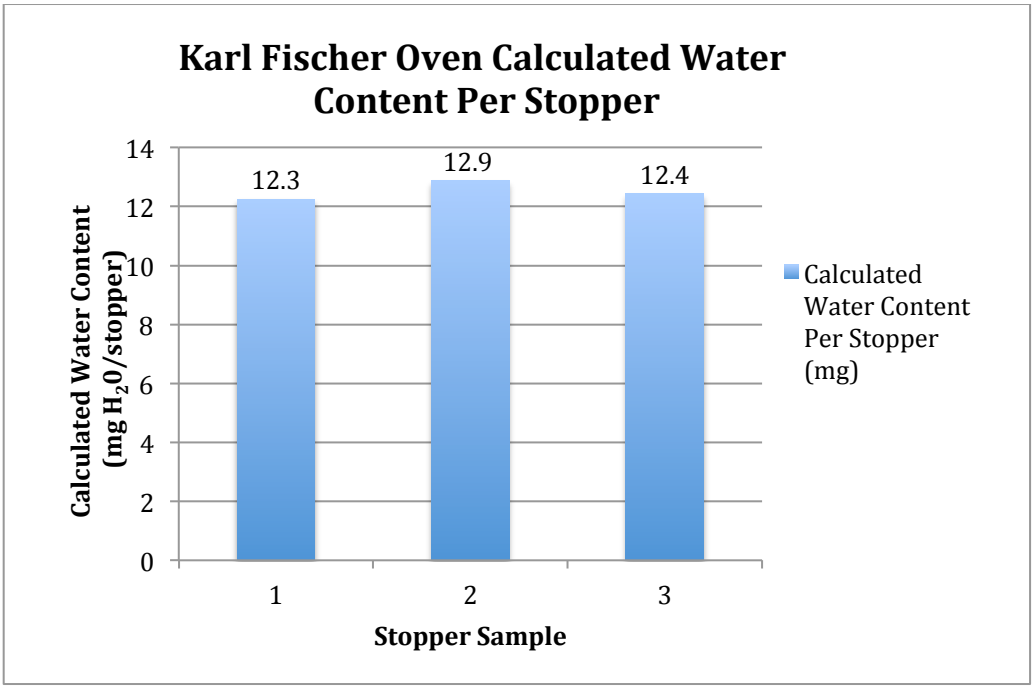

Figure 2.8: Karl Fischer Oven Calculated Water Content Per Stopper

3 Chapter 3: Karl Fischer Titration Method Utilizing THF Extraction

3.1 Background

3.1.1 THF Properties

Tetrahydrofuran (THF) is a clear, colorless liquid whose molecules weakly interact through Coulombic and dispersive forces at room temperature. This cyclic aliphatic ether molecule consists of one oxygen and four carbons, each saturated with two hydrogens, and exists in planar and nonplanar configurations [38]. THF is a low viscosity liquid which is completely miscible in water and exhibits a low freezing point, low boiling point, and high volatility [36]. Due to its molecular structure and physiochemical properties, THF has the capability to solvate both polar and nonpolar compounds and therefore is utilized in a wide range of applications. It also is stable in strongly basic conditions and can thus be used in specialty synthesis which involve complex catalysts and Grignard reactions [39]. THF may be utilized as a solvent for elastomers [36] (such as those used for rubber stoppers) and is soluble with chlorinated rubber, but not with fluorinated hydrocarbons such as PTFE [40]. THF reacts readily with oxygen to produce hazardous peroxides, especially in the presence of light, and therefore must be stored in a dark container with a nitrogen blanket. It is an irritant to the eyes, skin, and mucous membranes because the solvent has a defatting action, which leads to dehydration [36].

3.2 Materials

A Metrohm 756 Karl Fischer Coulometer with diaphragm-less generator electrode and Hydranal Coulomat AG analytical solution was used for all water content

determinations. Twenty millimeter West 4416/50 gray bromobutyl rubber single vent lyophilization stoppers were placed in 125-mL glass screw cap bottles and covered with tetrahydrofuran (THF) for extraction purposes. A VWR Orbital Mini Shaker was utilized to shake the samples. Data was acquired using Brinkmann Titrino Workcell 5.00 and Metrohm Tiamo 2.3 software. Reference Appendices for specific instrument parameters such as start drift, voltage, minimum extraction time, and stop drift. Parameters for Brinkmann Titrino Workcell 5.00 were equivalent to parameters outlined for Metrohm Tiamo 2.3 software.

3.3 Original Test Method

3.3.1 Analytical Method

Twenty millimeter stoppers were added to 125-mL sample bottles with septum caps and a graduated cylinder was used to aliquot 40 mL of tetrahydrofuran (THF) into the sample bottles. An additional 125-mL bottle with septum cap was prepared in the same fashion without the presence of a stopper to serve as the blank control. Samples and blank were placed on a shaker and shook for 4 hours at a speed of 270 rpm. Bottles were then set onto the lab bench to complete an extraction time of not less than 24 hours since initiation of sampling. In triplicate, 200-µL THF aliquots from each sample and the blank control were removed through the septum cap using a calibrated 250-µL syringe and were injected into the coulometric titrator to identify the water content of each aliquot. The coulometric cell was swirled after sample injection, and prior to titration initiation, to prevent residual sample from sticking to the sides of the cell and not being titrated. The amount of water in milligrams (mg) per stopper was then calculated per the following equation:

$$mg_{Water}/Stopper = \frac{\left(\overline{W}_t - \overline{W}_b\right)\left(V_t\right)\left(1000\right)}{\left(V_A\right)\left(N\right)\left(1000\right)}$$

$\overline{W}_t$	=	Mean weight of water titrated in the sample, in μg H_20
$\overline{W}_b$	=	Mean weight of water titrated in the blank, in μg H_20
V_t	=	Initial volume of solution, in mL
1000	=	Conversion to mL
V_A	=	Volume of each sample aliquot, in μL
N	=	Number of stoppers
1000	=	Conversion to mg

This original test method was derived from an unpublished moisture content validation study performed at Abbott Laboratories for elastomeric closures in 2002 [35].

3.3.2 Results/Discussion

In testing using the original Karl Fischer THF extraction analytical method, the water content results calculated for four equivalent stoppers were 7 mg, 9 mg, 10 mg, and 9 mg. This resulted in a standard deviation of 1.3 mg and a percent relative standard deviation (%RSD) of 14.4%. Using the same test method, additional testing was performed in which a bag of stoppers was stored in the laboratory and sampled and tested at 24-hour intervals. As shown in Table 3.1, this testing also gave variable results with relatively high standard deviations and %RSD.

Table 3.1: Calculated Water Content Using Original THF Extraction Test Method

Sample	Sample 1 (mg H2O /stopper)	Sample 2 (mg H2O /stopper)	Sample 3 (mg H2O /stopper)	Sample 4 (mg H2O /stopper)	Mean (mg H2O /stopper)	Standard Deviation (mg H2O /stopper)	% RSD
0 Hr.	7	8	8	7	8	0.6	8.4
24 Hr.	10	12	11	13	12	1.3	10.8
48 Hr.	9	12	9	5	9	2.8	32.8
72 Hr.	6	7	11	9	8	2.1	25.4

3.4 Test Method Assessment

3.4.1 Analytical Method

In order to isolate the possible sources of test method variability in the THF extraction method, all method assessment experiments were performed without the presence of a stopper unless stated otherwise. This eliminated the possible variability that could be attributed to the stopper samples themselves. Tested samples consisted solely of THF to mimic the blank in the original analytical method.

3.4.1.1 Sample Container

Cap Type

A graduated cylinder was used to aliquot 40 mL of tetrahydrofuran (THF) into nine 125-mL sample bottles. Onto three bottles, thick septum caps were added, three more bottles had thin septum caps, and the final three bottles received non-septum caps. The extraction and test process remained the same as the original test method except samples were tested at 4, 10, 24 and 32 hours and the non-septum cap samples required the removal of the cap for sample injection aliquoting.

Image 3.1: Septum Caps and Non-Septum Caps

In a second experiment, a graduated cylinder was used to aliquot 40 mL of tetrahydrofuran (THF) into seven 125-mL sample bottles and non-septum caps were added. The extraction and test process remained the same as the original test method except samples were tested at 0, 24 and 36 hours and at each of the test intervals, for three of the samples, a thick septum cap was added through which sample injection aliquots were obtained. For the other samples, the non-septum caps were removed in a dry box to obtain the sample injection aliquots. The samples with septum caps only had exposure to the septum caps during the sample injection testing periods and during the rest of the extraction time, non-septum caps were present on these samples.

Sample Bottle

Six 125-mL bottles and PTFE-lined non-septum caps were removed from the laboratory cabinets where they were stored at ambient conditions after washing and drying. Three of the bottles and caps were placed in a 100^{o}C dry heat oven to

further dry for one hour and then were cooled in a desiccator for another hour. Next, a pipette was used to aliquot 40 mL of tetrahydrofuran (THF) into all of the sample bottles and the caps were added. The extraction and test process remained the same as the original test method except samples were tested at 0, 24, and 48 hours and for all samples, the non-septum caps were removed in a dry box to obtain the sample injection aliquots and the coulometric cell was not swirled prior to titration initiation.

3.4.1.2 Equipment for Measurement

Three 40-mL samples of THF were aliquoted into 125-mL bottles with non-septum caps using a funnel and graduated cylinder. An additional three 40-mL samples of THF were aliquoted into 125-mL bottles with non-septum caps using a glass pipette. The extraction and test process remained the same as the original test method except samples were tested at 0, 24 and 33 hours and for all samples, the non-septum caps were removed in a dry box to obtain the sample injection aliquots.

3.4.1.3 Location of Sample Injection Aliquoting

Six 40-mL samples of THF were aliquoted into 125-mL bottles with non-septum caps using a glass pipette. The extraction and test process remained the same as the original analytical method except samples were tested at 0, 6, 10, 24, and 31 hours and no swirling of the titration cell occurred after sample injection. Three of the samples' injections were aliquoted by removing the non-septum caps in a dry box. The other three samples' injections were aliquoted by removing the non-septum caps on a lab bench.

3.4.1.4 Titration Methodology

Three 40-mL samples of THF were aliquoted into 125-mL bottles with non-septum PTFE-lined caps using a glass pipette. The samples were then analyzed twice at time zero using the same test process as the original test method except the caps were removed in the dry box to obtain the sample injection aliquots. In addition for the first test, the titration cell was swirled between each sample, but no shaking occurred after sample injection and prior to titration initiation. For the second test, the titration cell was swirled between each sample and after each sample injection prior to titration initiation.

3.4.1.5 Extraction Process

Duration of Shaking

Six stoppers were each placed in a 125-mL bottle with PTFE-lined cap and covered with 40 mL of THF aliquoted using a glass pipette. Three of the stopper samples were placed on a shaker and shook for a total of 8 hours while the other three stopper samples remained on the lab bench for the 8 hour period. Both sets of stoppers were tested at zero, 2, 4, 6, and 8 hours. Shaker stopper samples were removed from the shaker one at a time for testing to ensure the maximum amount of time for each stopper on the shaker over the eight hours. The test process remained the same as the original analytical method except the non-septum caps were removed in the dry box to obtain the sample injection aliquots and no swirling of the titration cell occurred after sample injection and before titration initiation.

Length of Extraction

Three stoppers were each placed in a 125-mL bottle with PTFE-lined cap and covered with 40 mL of THF aliquoted using a glass pipette. A second set of three bottles was prepared in the same fashion without the presence of stoppers to serve as the blank controls. The extraction and test process remained the same as the original test method except samples were tested at 0, 6, 10, 24, and 31 hours. In addition, the non-septum caps were used and removed in the dry box to obtain the sample injection aliquots and no swirling of the titration cell occurred after sample injection and before titration initiation.

3.4.2 Test Method Assessment Results/Discussion

3.4.2.1 Sample Container

Cap Type

As shown in Figure 3.1, samples with a thin septum cap had the greatest water content gain over time, followed by the thick septum cap samples, and finally the non-septum cap samples. It should be noted that sample #2 of the thin septum caps had a water content of 118.7 µg/200 µL at 4 hours and increased up to 312.4 µg/200 µL by 32 hours. Due to this extremely large amount of water, this sample's results were not graphed. The standard deviation for the thin septum cap samples started at 47.9 µg at 4 hours and increased up to 141.8 µg at 32 hours. The standard deviations ranged from 1.6-4.3 µg for the thick septum cap samples and 0.6-3.3 µg for the non-septum cap samples over the same time period. The average µg of water per sample at 32 hours of extraction was 149.6 µg/200 µL for the thin septum caps, 57.0 µg/200 µL for the thick septum caps, and 29.1 µg/200 µL for the non-septum

cap samples. Based on this experiment, it was concluded that not only the presence of a septum impacted the water content results of a sample during the extraction period, but also the type of septum used was an influence. Additionally, the use of thin septum caps should be avoided as they have an extreme amount of variability in terms of their contribution to the water content results.

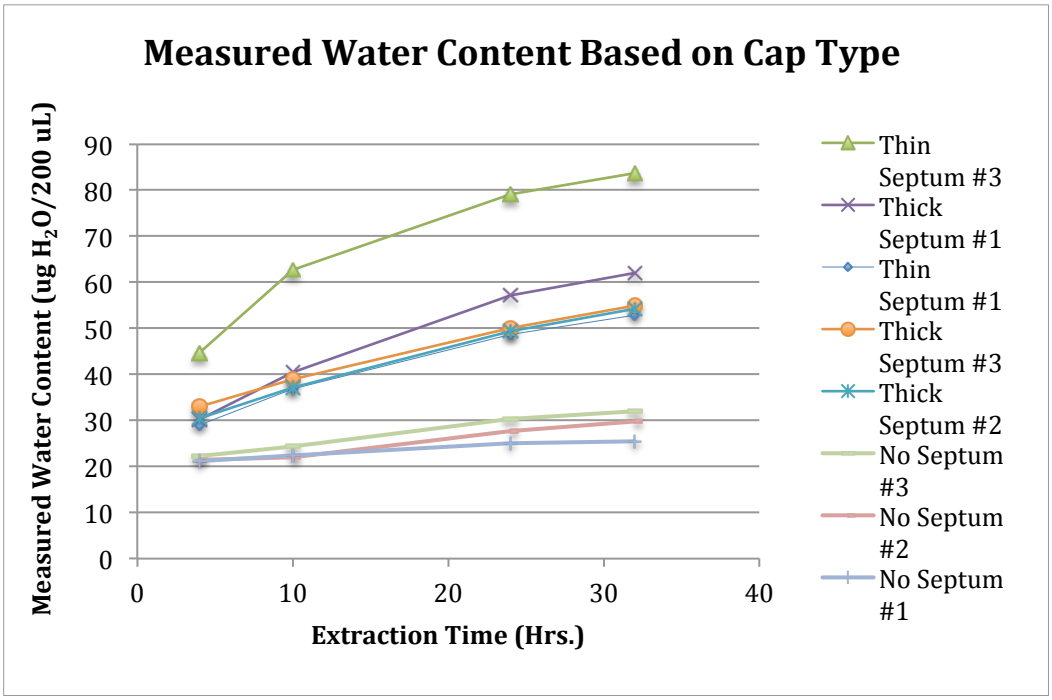

Figure 3.1: Measured Water Content Based on Cap Type

In the second experiment in which a subset of samples were exposed to thick septum caps only during the sample injection periods, results again showed an influence by the septum caps on the sample moisture content results. As shown in Figures 3.2 and 3.3, the water content of the samples tested using septum caps had a greater increase over the 36 hour extraction time than the water content of samples that were only exposed to the non-septum caps. For the septum cap samples, the mean water content increased from 36.5 µg water/200 µL at time zero to 45.3 µg

water/200 µL at 36 hours. For the non-septum cap samples, the mean water content increased from 36.8 µg water/200 µL at time zero to 39.6 µg water/200 µL at 36 hours. Therefore, the use of a septum, even for a short period of time, still impacted the samples' water content. The THF sample was in direct contact with the septum for approximately a minute during syringe rinsing and sample aliquoting. Even this brief exposure and contact time caused a higher water content sample result during this experiment. Finally, one sample with a non-septum cap was not included in the data graphed due to its high water content. Results for this sample were 40.1 µg/200 µL at time zero, 57.7 µg/200 µL at 24 hours and 64.7 µg/200 µL at 36 hours. Upon comparison of this sample's non-septum cap to the other non-septum caps, it was found that the lining of the cap was bulging and was a slightly different color than the other caps' linings. Therefore, not only the presence of a septum, but even the composition of the non-septum cap lining impacted the water content results of the samples.

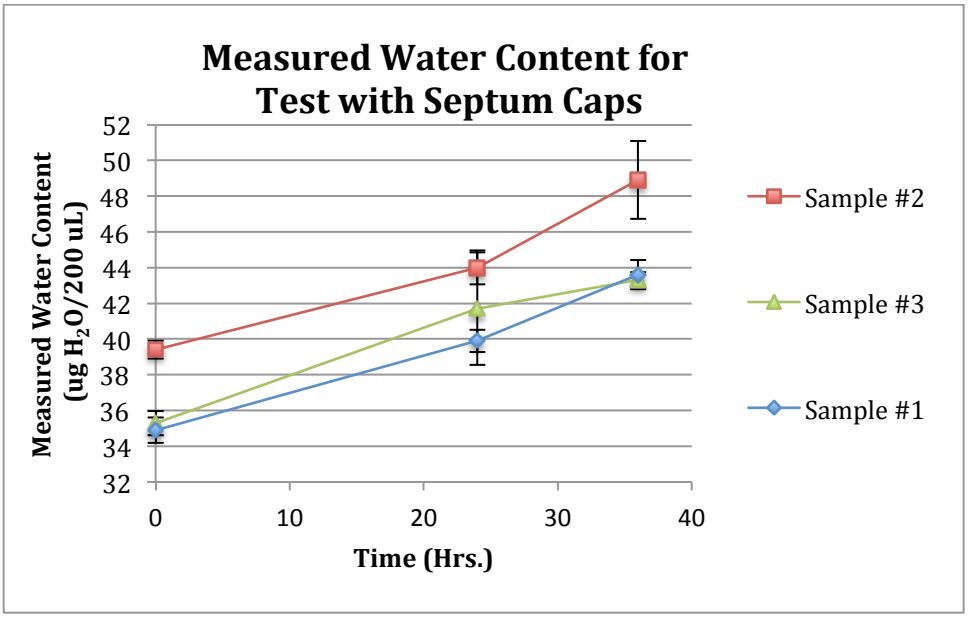

Figure 3.2: Measured Water Content for Test with Septum Caps

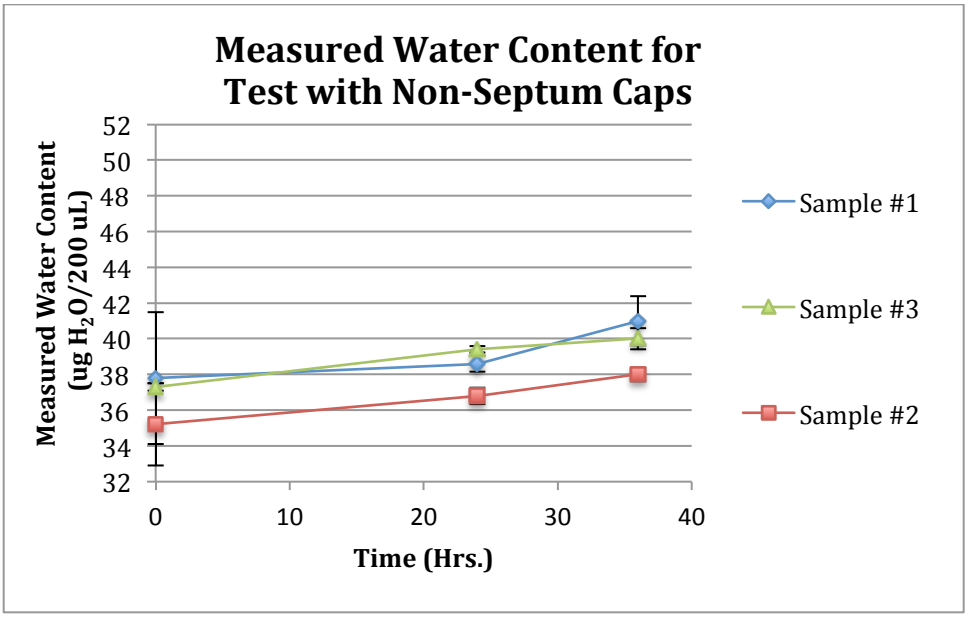

Figure 3.3: Measured Water Content for Test with Non-Septum Caps

Sample Bottle

As shown in Figure 3.4, additional drying of the sample bottles and PTFE-lined caps did not impact the water content of a THF sample in amount or variability. At time zero, there was a minor difference of approximately 2 micrograms (µg) in water

content between the two sets of samples, but by the 24 and 48 hour time points, there was no significant difference between the oven dried sample container samples and the not oven dried sample container samples. The sample containers and caps were thereby at a consistent equilibrium with the environment during storage that the oven drying and cooling did not alter. They therefore did not have a significant impact on the amount or variability of the measured water content of samples placed within them.

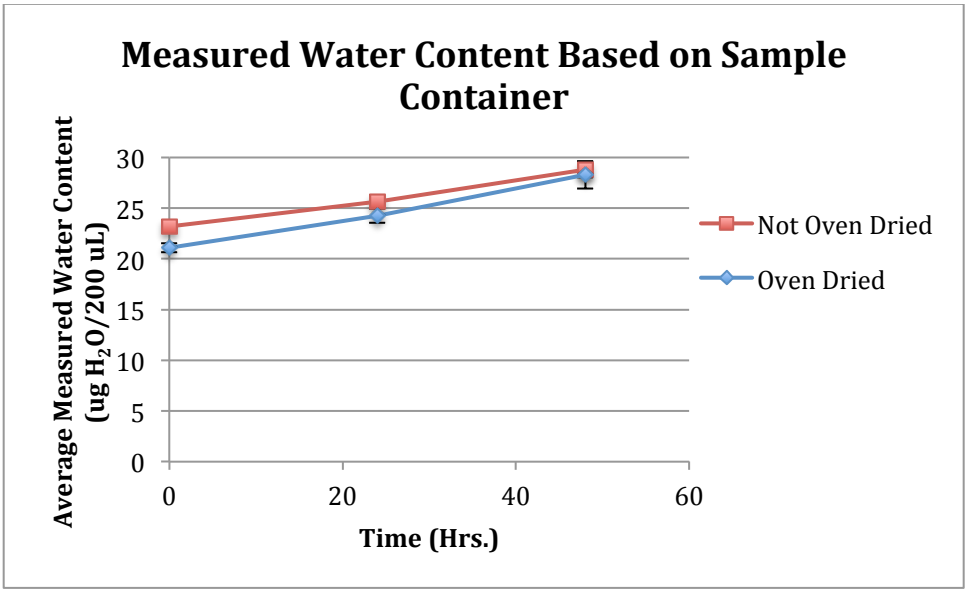

Figure 3.4: Measured Water Content Based on Sample Container

3.4.2.2 Equipment for Measurement

The use of a glass pipette for sampling the THF into the sample bottles achieved a lower water content than sampling with a funnel and graduated cylinder, and as well, a smaller standard deviation was recorded at each tested time point, as shown in Figure 3.5. It was theorized that these results were due in part to the fact that the pipette pulled samples from the bottom of the THF bottle from solution that was not

directly exposed to the atmosphere and thus the air/solvent interface was minimal. THF samples aliquoted with a graduated cylinder on the other hand were poured through a funnel and into a graduated cylinder thereby allowing increased exposure to the atmosphere with mixing thus a larger air/solvent interface and therefore inconsistent water absorption from the environment. In addition, the residual water on the interior surface of the pipette, graduated cylinder, and funnel may have been another contributing factor. All of this glassware was washed, dried, and stored in the same manner. These pieces of equipment therefore had the same exposure to moisture in the environment. However, the residual water on each surface may have varied slightly. Furthermore, the THF samples aliquoted with a graduated cylinder may have had contact with a greater amount of residual water because the solution traveled across the interior surface of both the funnel and the graduated cylinder. The THF samples aliquoted with a pipette conversely were only exposed to the interior surface of the glass pipette.

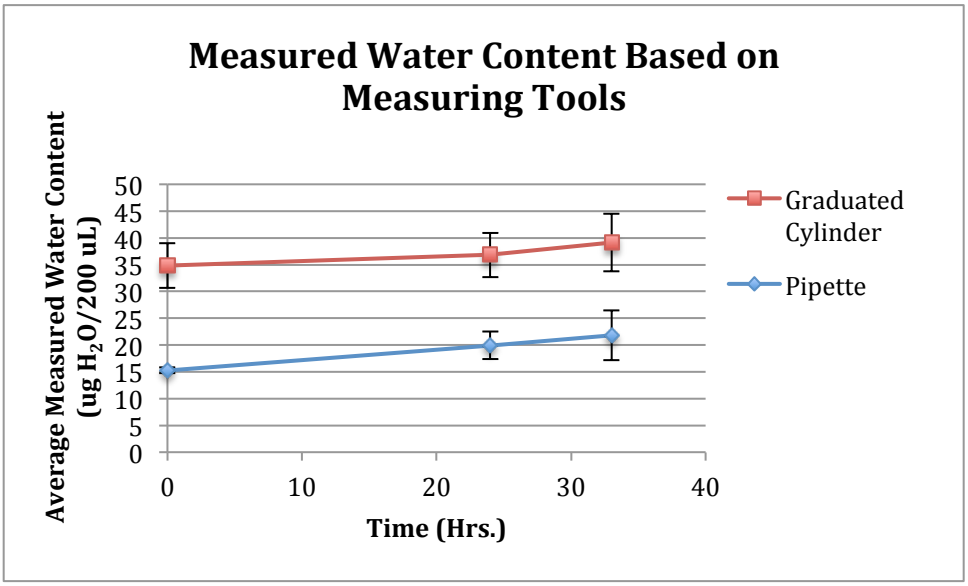

Figure 3.5: Measured Water Content Based on Measuring Tools

3.4.2.3 Location of Sample Injection Aliquoting

Sample injections aliquoted in a dry box had a lower water content over time compared to sample injections aliquoted on the lab bench, as shown in Figures 3.6 and 3.7. Sample to sample variance was slightly higher with the samples aliquoted in the dry box, but injection-to-injection variance was lower with these samples. It was therefore concluded that the exposure of the THF samples to the lab atmosphere during aliquoting on the lab bench influenced the samples' water content. It led to increased injection-to-injection variability of a single sample and a greater increase in samples' water contents over time. Because sample to sample variance was higher with sample injection aliquoting in the dry box, for future testing using a dry box, it would be good laboratory practice for one to utilize greater than one blank and calculate a water content average to ensure a representative blank water content result.

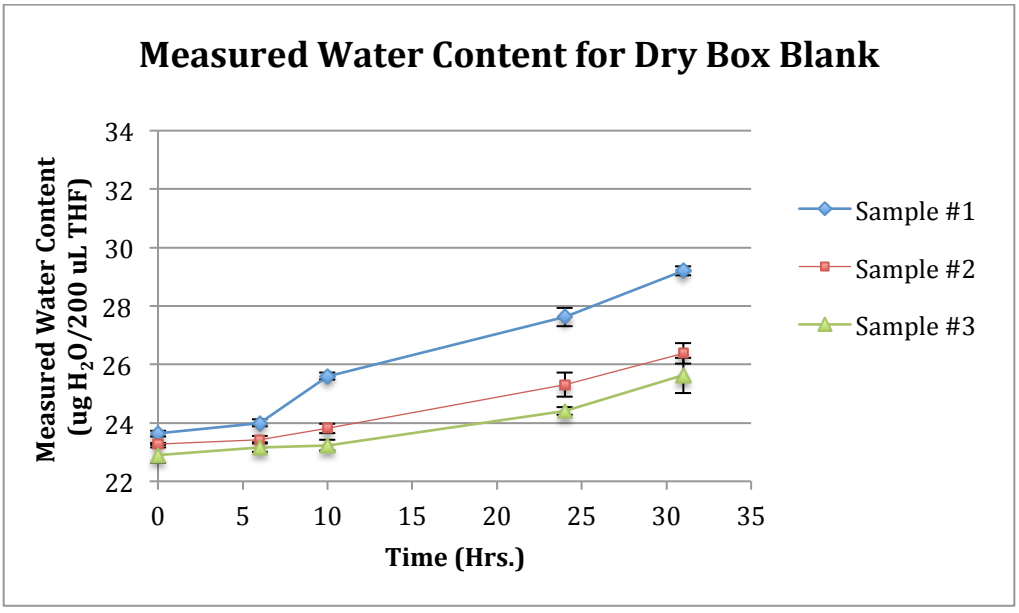

Figure 3.6: Measured Water Content for Dry Box Blank

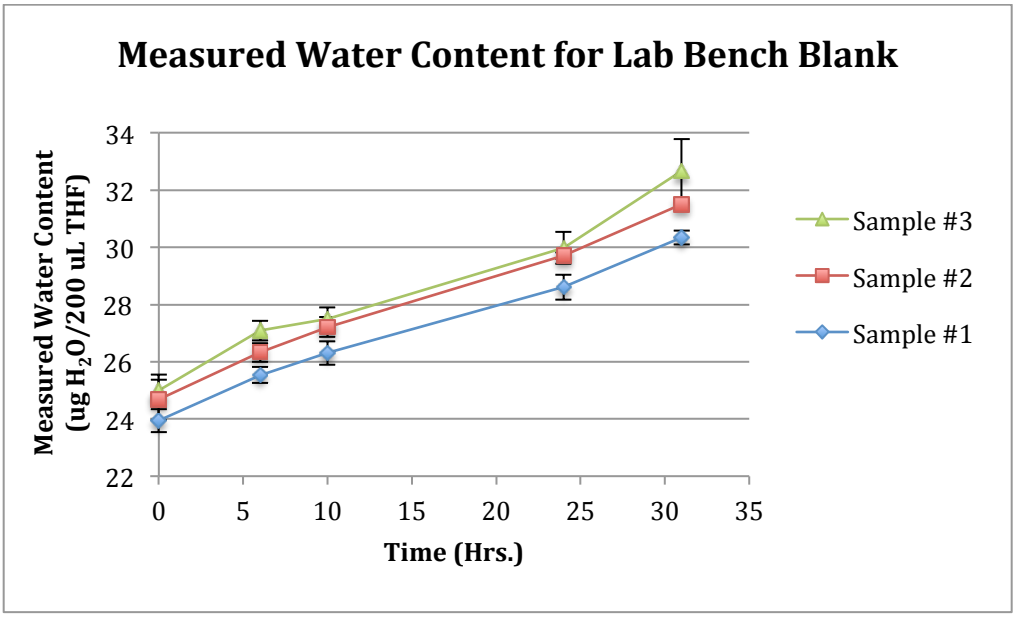

Figure 3.7: Measured Water Content for Lab Bench Blank

3.4.2.4 Titration Methodology

Due to the design of the titration cell, it was a standard practice to swirl or shake the cell during a test session to disrupt and remove the pockets of water that were not reached during the routine stirring of the solution. In addition, this practice collected the water that was stuck to the sides of the vessel. After injecting a sample, it was also standard to swirl the cell to ensure the entire sample got titrated. However, during experimentation it was found that the agitation of the titration cell caused the instrument drift to increase as additional water within the system had to be titrated. As seen in the results documented in Figure 3.8, the use of swirling or shaking after sample injection caused large injection-to-injection variance, sometimes as great as 20 µg. It was concluded that when samples have a higher water content, then a 20 µg variance does not largely impact the calculated water content of the sample. However, in the cases where the sample's water content is

low, such as less than 100 µg, then a 20 µg variance greatly alters the calculated water content, which is reflected in large sample to sample variance as well.

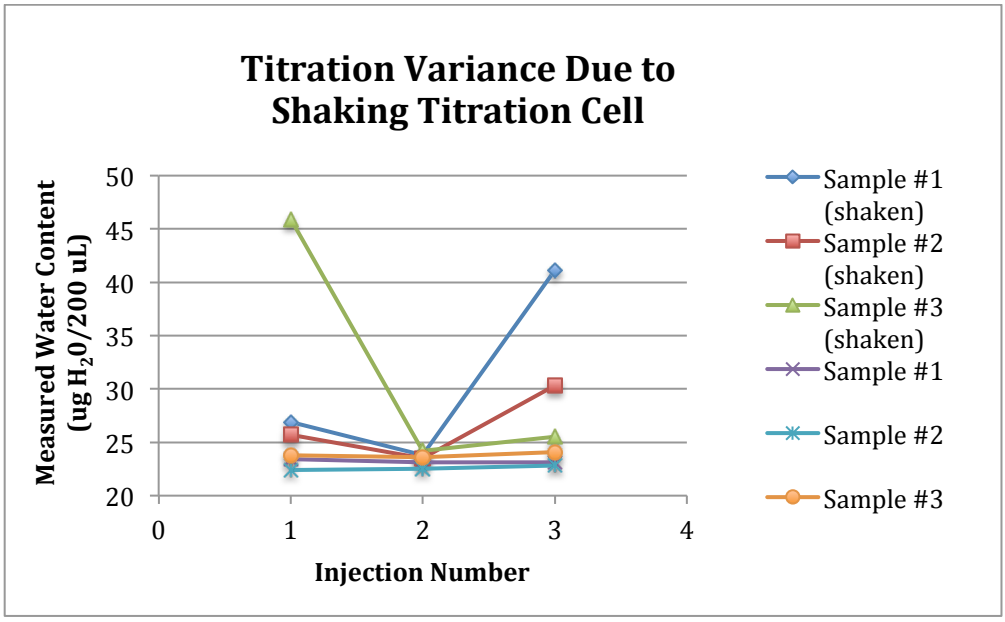

Figure 3.8: Titration Variance Due to Shaking Titration Cell

3.4.2.5 Extraction Process

Duration of Shaking

The initial stopper extraction method specified a four hour period during which stopper samples were shaken on a mechanical shaker followed by not less than 20 additional hours of extraction on the lab bench to complete the extraction process. However, based on the data shown in Figures 3.9 and 3.10, the shaking period was not a critical feature in the extraction process. The stoppers that were not shaken had a slightly higher calculated water content at each interval until the 8 hour time point. It was hypothesized that this was not due to the lack of shaking, but instead the result of the order in which the samples were tested, as this set of samples was tested after the shaken set. This data therefore showed that the shaking did not

increase or speed up the extraction capabilities of the THF solution. The diffusion of the water through the rubber stopper to its surface was the rate-limiting step in the extraction process, not the diffusion of the water away from the stopper surface into the THF solution. This stopper extraction method was initially developed to test multiple stoppers within a single sample of THF solution, so the period of shaking may have increased importance with additional stoppers within the extraction fluid to ensure all stopper surface areas have constant exposure to the THF solution. It was noted that the shaken samples had a higher standard deviation from injection to injection at time zero. This suggested that the shaking did cause increased disturbance and possible pockets of water were extracted into the THF solution, but ultimately the solution became homogenous as the standard deviations were lower at the other time points.

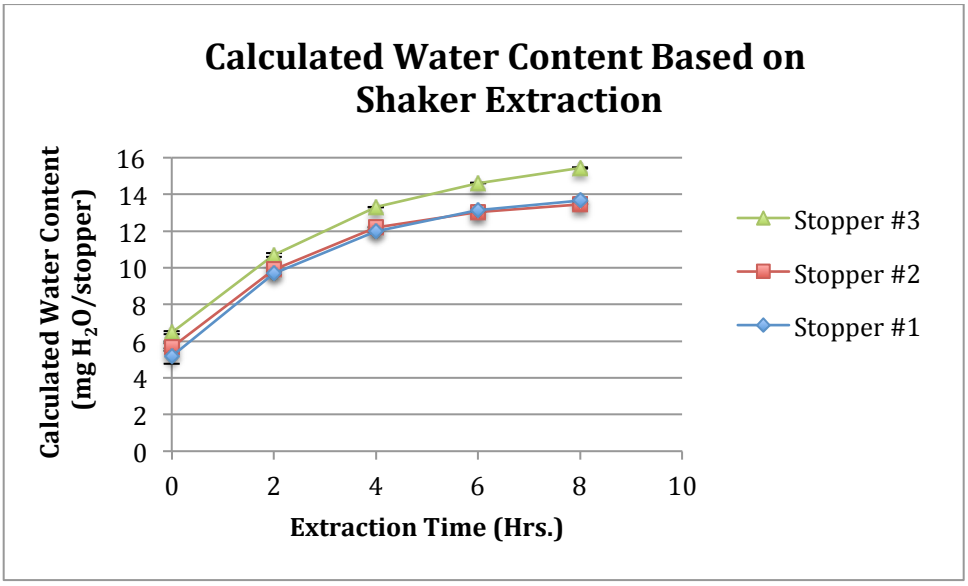

Figure 3.9: Calculated Water Content Based on Shaker Extraction

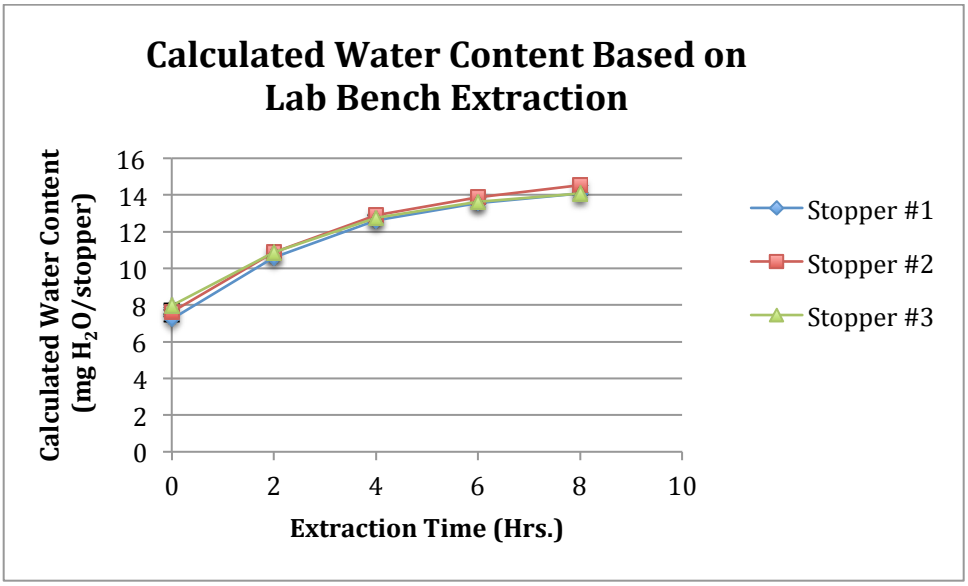

Figure 3.10: Calculated Water Content Based on Lab Bench Extraction

Length of Extraction

As shown in Figure 3.11, the bulk of the water extracted from the stopper samples occurred within the first 6 hours of the extraction period. After this time point, small amounts of water continued to be extracted until the conclusion of the experiment. However by 6 hours, 93% of the water measured over the length of the experiment had been extracted. A 24 hour extraction period was therefore four times longer than required to identify the bulk of the water in the stopper sample and ensured the extraction rate was nearing zero at conclusion of the test.

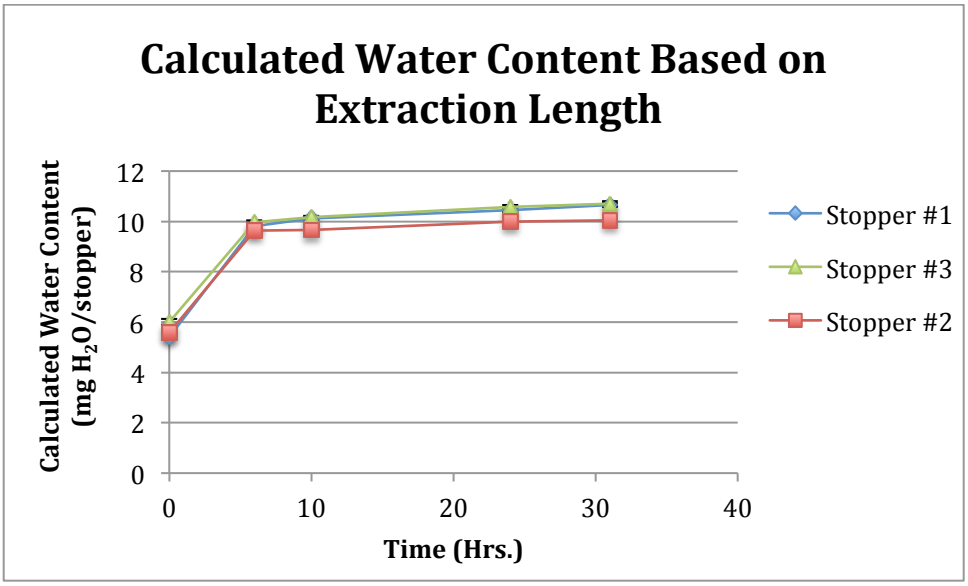

Figure 3.11: Calculated Water Content Based on Extraction Length

3.5 Final Test Method

3.5.1 Analytical Method

Based on the results from the test method assessment experiments, the finalized test method is as follows.

A 20-mm stopper is added to a 125-mL sample bottle with non-septum PTFE-lined cap. It is recommended the glassware and in particular the caps are utilized only for stopper moisture testing to minimize exposure of the glass, cap, cap liner, and liner adhesive to other chemicals which may alter the components and lead to future water content variability when used for moisture testing. To reduce the exposure of the THF to the atmosphere, a glass pipette is used to aliquot 40 mL of THF into the sample bottle containing the stopper. Three additional 125-mL bottles with non-septum PTFE-lined caps are prepared in the same fashion without the presence of a stopper to serve as the blank controls. These multiple blanks help to account for

sample to sample variance that is not a reflection of the water content of the stopper samples themselves. For the extraction process, the sample and blanks extract on the lab bench until not less than 24 hours has elapsed since initiation of sampling to ensure the extraction rate is close to zero. After removing the PTFE-lined cap in a dry box, 200-µL THF aliquots are taken using a calibrated 250-µL syringe from each sample and the blanks in triplicate. Aliquots are injected into the center of the coulometric titrator to identify the water content of each injection. The coulometric cell is swirled between samples to remove any residual water from the sides of the cell and break up any pockets of water, but not after sample injection and prior to titration initiation as this causes a higher drift, which leads to wide injection-to-injection variance. The aliquot's water content is then titrated using the Karl Fischer coulometric titration method until the specified ending criteria are met. The amount of water in mg per stopper sample is then calculated per the following equation:

$$mg_{Water}/Stopper = \frac{\left(\overline{W}_t - \overline{W}_b\right)\left(V_t\right)\left(1000\right)}{\left(V_A\right)\left(N\right)\left(1000\right)}$$

$\overline{W}_t$ = Mean weight of water titrated in the sample, in µg H_20

$\overline{W}_b$ = Mean weight of water titrated in the blanks, in µg H_20

V_t = Initial volume of solution, in mL

1000 = Conversion to mL

V_A = Volume of each sample aliquot, in µL

N = Number of stoppers

1000 = Conversion to mg

3.5.2 Final Test Method Results/Discussion

The calculated water content results for the three stoppers tested utilizing the final Karl Fischer titration method with THF extraction are shown in Figure 3.12. The mean calculated water content was 9.9 mg water/stopper +/- 0.8 mg. The injection-to-injection variability for each sample, as reflected by the error bars in the figure, was minimal with % RSDs at or below 1.1%. This indicated that the modifications made to the method properly reduced the injection-to-injection variance seen during initial testing. The three blanks had a water content of 28.6 µg/injection, 29.5 µg/injection, and 27.6 µg/injection, thus achieving a mean water content of 28.6 µg water/injection +/- 1 µg. The utilization of the average water content of the multiple blanks, instead of just one blank, during the sample water content calculations therefore lessened the possible skew in sample results due to external influences outside of sample to sample variance. As a whole, there was more variance than was expected in the results. However, as this method had not been performed in a number of weeks, it was hypothesized that analyst technique and not the method itself was the greatest driver of the variance.

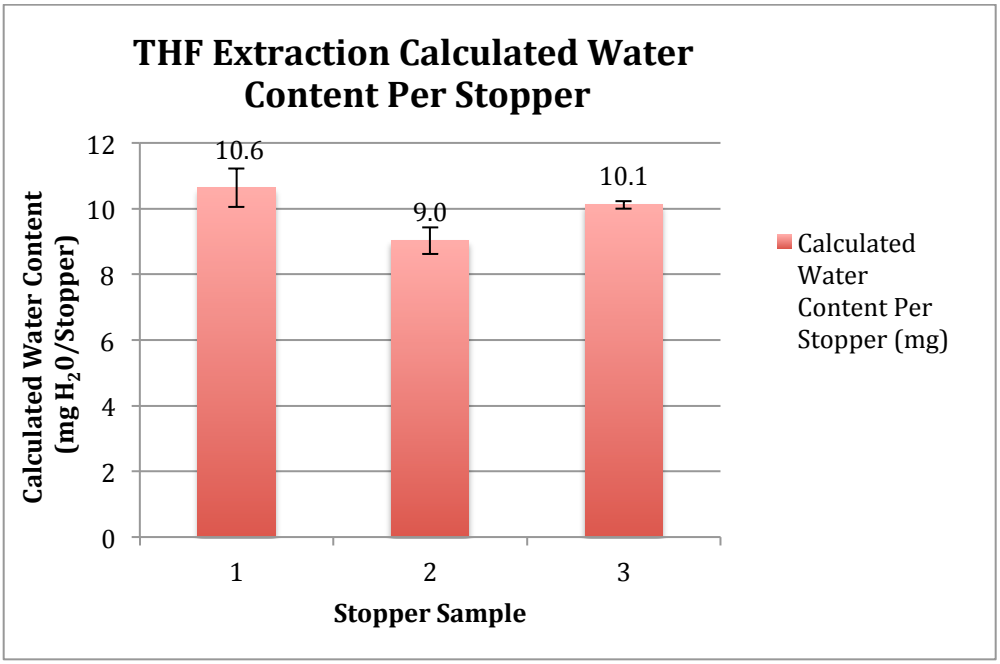

Figure 3.12: THF Extraction Calculated Water Content Per Stopper

4 Chapter 4: Test Method Comparison

4.1 Sample Preparation

For the Karl Fischer titration method utilizing an oven, the critical parameters during sample preparation are use of a dry box, proper evacuation of moisture from the sample vials prior to utilization, consistent sample cutting technique with capability to cut small samples, and immediate sealing of samples into sample vials. The sample preparation takes only 5-10 minutes once the sample vials have been dried, but due to the elasticity and flexibility of the rubber, obtaining a consistent shape and small sized stopper sample may be difficult.

For the Karl Fischer titration method utilizing THF extraction, the critical parameters are the type, composition, and dryness of the caps, cap liners, and bottles and the method of THF aliquoting. Seemingly minor variations such as the use of septum caps versus non-septum caps during the extraction or the use of a funnel and graduated cylinder versus a glass pipette for THF aliquoting can have significant implications on the measured water content. The stoppers are tested whole, therefore manipulation of the sample is minimal, but a 24 hour extraction time must occur prior to titration initiation.

4.2 Reagents/Equipment

Both the THF extraction method and the oven method utilize a Karl Fischer coulometric titrator for water content analysis with a Hydranal AG analytical solution containing the necessary Karl Fischer reaction components. They also involve the use of a dry box either for sample preparation or analysis. The THF extraction method utilizes PTFE-lined non-septum caps and 125-mL bottles that are

reusable as long as they are properly washed and dried and preferably dedicated to the THF extraction test method. In addition, tetrahydrofuran (THF) must be used for the extraction process and a calibrated 250-µL air-tight syringe delivers the sample aliquot injections. The oven method, on the other hand, uses disposable PTFE-backed silicone septa caps and 6-mL KF sample vials, and a capper that hermetically seals the vials. More critically, the oven method requires the use of a specialized oven with a specific needle through which a dry carrier gas is passed. The oven must have the proper components to join with the Karl Fischer coulometric titrator and may be expensive, especially if an autosampler is desired.

4.3 Analysis

At the time of analysis, the Karl Fischer titration method with oven requires little analyst intervention. Once the vial has been placed into the oven and the needle inserted, the analyst must only press start and the titration is performed. As it is through a direct connection between the oven and the Karl Fischer titration cell that the stopper moisture is introduced, sample addition variation and titration cell changes are minimized. However, this titration may take thirty minutes or greater depending on the sample and test parameters chosen.

The Karl Fischer titration method with THF extraction requires more manipulations and analyst input at the time of analysis. Analyst must swirl the titration cell prior to sample addition, wait for the drift to stabilize, and then inject the sample aliquots into the center of the titration cell. Analyst cannot swirl the titration cell after sample aliquoting or large injection-to-injection variation is recorded. This analytical method is therefore more sensitive to analyst technique and analyst-to-

analyst variation. Three aliquots must be injected per sample and the total analysis time is typically not more than fifteen minutes. Due to the sensitivity of the test method to septum cap moisture, the sample aliquots cannot be made through a septum, but instead the sample lid must be removed. This analytical method requires greater manipulation by the analyst and exposure to THF, which is volatile and flammable. However, all sample aliquoting is performed in a dry box, which lowers the safety concerns.

4.4 Calculations

The calculations for both the Karl Fischer titration method with oven and the Karl Fischer titration method with THF extraction are comparable. Each test method's calculations take into account the testing environment by subtracting out a blank and results are obtained in milligrams (mg) water per stopper. The oven method also calculates the percentage of water per stopper based on the initial weight of the entire stopper.

4.5 Test Method Development

The Karl Fischer titration method with oven must be specialized to each type and composition of stopper in terms of the sample shape, size, oven temperature and carrier gas flow rate. Small variations to any of these parameters will impact the moisture content results measured. In particular, experiment trials found that the diffusion path length of the water from the interior of the stopper sample to the surface is the rate-limiting step in identifying an accurate water content of the sample. Therefore test parameters must be identified for each type and

composition of stopper that will optimize the diffusion path length. This fact makes this analytical method more time consuming during development activities and could make it more difficult to validate.

For the Karl Fischer titration method with THF extraction, the testing parameters are not as specialized to the stopper characteristics, therefore the development time is shorter and less complex for a new stopper. The stopper is tested whole, so sample size and shape need not be evaluated. In addition, the THF extraction method's main variables are the THF extraction time and the extraction volume. As a large percentage of the stopper's water is extracted within the first six hours of extraction based on the performed trials, even if a new stopper required greater than six hours to remove most of the stopper's moisture, the 24-hour extraction time is likely to encompass this increased time. In addition, the THF extraction volume may need to be modified based on the size or theoretical water content of the stopper. However, this change is addressed in the calculations performed and does not largely modify the test method, therefore extensive development and revalidation is not necessary.

5 Chapter 5: Conclusion/Next Steps

This evaluation was focused on identifying the key parameters critical to each of the stopper moisture test methods, Karl Fischer titration method with oven and Karl Fischer titration method with THF extraction. Work thereby centered only on the analytical methods themselves and did not delve into how the key parameters may change or require revision when utilized for other stopper types beside the West 4416/50 gray bromobutyl single vent 20-mm stopper used in the above experiments. Future studies should survey the necessary modifications needed to use the oven or THF extraction method on other types of stoppers. In terms of stoppers for lyophilized products, there are two standard sizes used, 13 mm and 20 mm. One may hypothesis that the fraction of the stopper that should be used for the Karl Fischer oven method may only need to be specific to the two stopper sizes. However, additional exploration should be executed to identify if the stopper composition would also impact the mass and shape of sample to test. A further aspect to research for the oven method is a better technique for sampling stopper pieces. This may involve the development of a jig or acquisition of a specialize tool that can more easily cut through the flexible rubber while achieving smaller and more consistent sample sizes.

In focusing on the test methods themselves, both the Karl Fischer titration method utilizing an oven and the Karl Fischer titration method utilizing a THF extraction have their advantages and disadvantages. The Karl Fischer titration method with oven requires additional equipment-the oven and specialized needle and disposable seals and vials-which makes it a more expensive analytical method. However, it is

much less sensitive to sample preparation and analyst technique as long as the vials are evacuated of air, sample sizes and shapes are small enough, and preparation is performed in a dry box. The total time required for preparation and analysis is less than 2 hours and no analyst input is needed once the titration has been initiated. The oven method does require a longer development time, as it must be optimized for each stopper material, size, and shape. The proper sample mass, shape, oven temperature, and carrier gas flow rate must be identified for each new stopper type for testing.

The Karl Fischer titration method utilizing a THF extraction, on the other hand, uses standard equipment and materials typically found in a pharmaceutical analytical laboratory, thus it is less expensive to execute. The test method is very sensitive to the sample preparation, such as the bottles and caps used, as well as the THF aliquoting equipment, and therefore is more prone to sample to sample and analyst to analyst variation. The total time necessary for preparation and analysis is greater than twenty-four hours due to the obligatory extraction time. In addition, there is a greater amount of required analyst input during testing of the samples as the sample titration cell must be shaken prior to sample injection, but not after, and the analyst must manually inject the sample aliquots into the center of the cell. As there are fewer test variables for the THF extraction method, test development and optimization of the test method for a new stopper type is minimal and thus takes less time.

Finally, the Karl Fischer titration method with oven and the Karl Fischer titration method with THF extraction do not necessarily achieve equivalent results. The THF

extraction method is an absolute method where a large percentage of the stopper's water is extracted from the stopper over the first 6 hours of the extraction and an even greater percent is extracted by the completion of the 24 hour extraction period. This test method therefore does not have the capability to consistently extract only a small percentage of the stopper's water. However, the Karl Fischer oven method is more particular to the sample type, size, shape, and oven temperature used and so the percentage of water measured, relative to the total amount of water in the stopper, varies with the chosen parameters.

Beyond the realm of the stopper moisture test methods, there exists a broader topic to be addressed by pharmaceutical companies that manufacture lyophilized products. This topic is to identify the amount of moisture in a rubber stopper that will actually be able to interact with the lyophilized product and thus the extent to which the total water content of the stoppers needs to be known. Based on the lyophilized product's container closure design alone, it is understood that direct contact with the interior vial environment and thus the lyophilized product only occurs by a portion of the stopper (the interior of the legs and the bottom of the flange). Wang et al. state that 60% of the total stopper surface area is a product contact surface, while Donovan et al. cite approximately 50% of the stopper surface is exposed to the product per the stopper vendor data for the stoppers they used [7, 34]. Donovan et al. also concluded that approximately 50% of the total stopper moisture lost from the stopper over time at 25^{o}C and 40^{o}C was gained by the lyophilized product [7]. A given lyophilized product will thereby rarely be exposed to the entire moisture content of its stopper.

In addition, the standard storage temperature for lyophilized products is either 25°C or 2-8°C. As seen in the Karl Fischer titration method with oven, the amount of water released by the stopper is extremely small at temperatures below 100°C. It requires much higher temperatures for the stopper to release the amount of water one sees in both the final THF extraction method as well as the final oven method. However, it is acknowledged that the Karl Fischer titration testing is performed over a very brief interval of time under accelerated conditions to promote the release of the stopper's water. Ultimately, it is the amount of water released or absorbed by the stopper over the entire product shelf life at the specified storage conditions that is most critical.

Therein, the percentage of stopper moisture an analyst chooses to measure and thus the test method utilized is at his or her discretion based on knowledge of the drug product being tested and the manufacturing process and controls in place.

The above research has evaluated the Karl Fischer titration method with oven and the Karl Fischer titration method with THF extraction and identified the critical testing parameters and advantages and disadvantages of each analytical method. Other areas to explore have been identified and the bigger picture has been addressed. Product properties, storage conditions, stopper characteristics, and manufacturing controls may lend themselves to a particular stopper moisture test method. However, if the test method is validated and scientifically defendable when applied to the product in question, both the Karl Fischer titration method with oven or the Karl Fischer titration method with THF extraction may be suitable for water

content determination of rubber stoppers utilized for sealing lyophilized pharmaceutical products.

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Appendices

Karl Fischer Titration With Oven Blank Method

License ID	3665614	Program version	tiamo 2.3 - 98 - 1
Computer name	MPWMJRGXH3		
User	10130396		2013-04-106:35:37 AM UTC-

Method parameters

Method . . . . . KF Oven Blank Research Project
Method saving date 2012-10-26 08:43:38 UTC-5
Method version 4
Method group . . . . . Research Methods
Method status . . . . . original
Method saved by (full name) . . . . . Laura Voth
Method saved by (short name) 10130396

START **Main track**

General

Workplace view
Current view . . . . . on
Track view for live window
Live display 1 . . . . . Coulometric KF titration
Live display 2 . . . . . Main track
Statistics . . . . . on
Number of single determinations 3
Conditioning
Automatic conditioning . . . . . on

Application note

Method variables

Name	Type	Assignment	Fixed value	Comment	Monitoring
Sample Name	Text	ID1		Sample identification 1	off

Name . . . . . **Sample Name**
Type . . . . . Text
Assignment . . . . . on. ID1
Fixed value . . . . . off. . . . . .
Check at start . . . . . on
Comment . . . . . Sample identification 1

CALL **CALL coulometric KFT**

Call text	Track name	Condition	Condition
Start KFC	Coulometric KF titration	off	

REQUEST **REQUEST**

Sample data request
Sample position . . . . . off
ID1 . . . . . on
ID2 . . . . . off

Page 1 of 4

ID3 . . . . . off
ID4 . . . . . off
ID5 . . . . . off
ID6 . . . . . off
ID7 . . . . . off
ID8 . . . . . off
ID9 . . . . . off
ID10 . . . . . off
ID11. . . . . off
ID12 . . . . . off
ID13 . . . . . off
ID14 . . . . . off
ID15 . . . . . off
ID16 . . . . . off
Sample size . . . . . off
Unit . . . . . off
Message . . . . . off

TRACK **Coulometric KF titration**

Return immediately . . . . . on
Delete old data . . . . . off

KFC **KFC**

General/Hardware

Device

Device name . . . . . 831_1

Sensor

I(pol) . . . . . 10 µA
Electrode check . . . . . on

Cell

Generator type . . . . . without diaphragm
Generator current . . . . . 400 mA

Stirrer

Switch on/off automatically . . . . . on

Start conditions

Pause

Pause . . . . . 0 s

Control parameters

End point

EP at . . . . . 50.0 mV

Titration rate

Titration rate . . . . . optimal

Stop criterion

Stop criterion . . . . . rel.drift
Relative stop drift . . . . . 5 µg/min

Titration parameters

Extraction time	300	s
Temperature	25.0	°C
Time interval measuring point	2	s

Stop conditions

Stop time	off	s

Conditioning

Conditioning	on	
Start drift	20	µg/min
Drift correction	automatic	
Stabilizing time	0	s
Automatic start after sample addition time	off	
Manual start after sample addition with [Continue]	on	
Only start titration by a start command from a SEND command	off	
Show measured value during conditioning	off	

Additional evaluations

Fixed endpoint evaluation	off

Additional measured values

Additional calculated measured values	off
Additional external measured values	off

EXIT **Exit track**

CALC **CALC**

Result name	Formula	Unit	Decimal places	Assignment	Statistics
KFC OVEN BLANK	='KFC.EP.QTY'	ug	2	RS01	on
AVERAGE KFC OVEN BLANK	='RS.KFC OVEN BLANK.MNV'	µg	2	RS02	off

Result name	**KFC OVEN BLANK**
Formula	='KFC.EP.QTY'
Unit	ug
Decimal places	2
Assignment	RS01
Statistics	on
Description	
Result monitoring	off
Save result as common variable	off
Common variable	KFC OVEN BLANK
Save result as titer	off
Solution name	20 mL 807 buret

Result name	**AVERAGE KFC OVEN BLANK**
Formula	='RS.KFC OVEN BLANK.MNV'
Unit	µg
Decimal places	2
Assignment	RS02
Statistics	off
Description	
Result monitoring	off
Save result as common variable	on
Common variable	Average KFC OVEN BLANK
Save result as titer	off
Solution name	20 mL EU

REPORT **REPORT**

Report template

Report template	Research Report

Report output

Printer	off
PDF file	on
PDF file	F:\SH\PD\EMPLOYEE FOLDERS\Voth, Laura\Tiamo pdfs\Reports.pdf
Send e-mail	off
Mail to	

DATABASE **DATABASE**

Database

Research Project Database

Karl Fischer Titration With Oven Stopper Sample Method

Method parameters

Method . . . . . KF Oven Stopper Research Project
Method saving date 2012-10-26 09:11:10 UTC-5
Method version 4
Method group . . . . . Research Methods
Method status . . . . . original
Method saved by (full name) . . . . . Laura Voth
Method saved by (short name) 10130396

START **Main track**

General

Workplace view
Current view . . . . . on
Track view for live window
Live display 1 . . . . . Coulometric KF titration
Live display 2 . . . . . Main track
Statistics . . . . . off
Conditioning
Automatic conditioning . . . . . on

Application note

Method variables

Name	Type	Assignment	Fixed value	Comment	Monitoring
Name	Text	ID1		Sample identification 1	off
Lot #	Text	ID2		Sample identification 2	off
Weight of Stopper Sample	Number	Sample size		Sample size in mg	off
Sample size unit	Text	Sample size unit		Sample size unit	off
Weight of Entire Stopper	Number	ID3		in mg	off

Name . . . . . **Name**
Type . . . . . Text
Assignment . . . . . on ID1
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . . Sample identification 1

Name . . . . . **Lot #**
Type . . . . . Text
Assignment . . . . . on ID2
Fixed value . . . . . off . . . . .
Check at start . . . . . on

Comment . . . . . Sample identification 2

Name . . . . . **Sample size unit**
Type . . . . . Text
Assignment . . . . . on . . . . . Sample size unit
Fixed value . . . . . off . . . . . .
Check at start . . . . . on
Comment . . . . . Sample size unit

Name . . . . . **Weight of Stopper Sample**
Type . . . . . Number
Assignment . . . . . on . . . . . Sample size
Fixed value . . . . . off . . . . . .
Check at start . . . . . on
Comment . . . . . Sample size in mg
Variable monitoring . . . . . off
Lower limit . . . . . .
Upper limit . . . . . .
Message . . . . . .
Display message . . . . . on
Record message . . . . . on
Message by e-mail . . . . . off
Use e-mail template . . . . . off
E-mail template . . . . . .
Mail to . . . . . .
Subject . . . . . Message from tiamo - Method 'Coulometric KF titration_1' - Command 'Main track'
User . . . . . .
Mail from . . . . . .
SMTP Server . . . . . .
POP3 Server . . . . . .
Acoustic signal . . . . . off
Action . . . . . off
Cancel determination . . . . . on
Cancel determination and series . . . . . off

Name . . . . . **Weight of Entire Stopper**
Type . . . . . Number
Assignment . . . . . on ID3
Fixed value . . . . . off . . . . . .
Check at start . . . . . off
Comment . . . . . in mg
Variable monitoring . . . . . off
Lower limit . . . . . .
Upper limit . . . . . .
Message . . . . . .

Display message . . . . . on
Record message . . . . . on
Message by e-mail . . . . . off
Use e-mail template . . . . . off
E-mail template . . . . .
Mail to . . . . .
Subject . . . . . Message from tiamo - Method 'KF Oven Sample Research Project' - Command 'Main track'
User . . . . .
Mail from . . . . .
SMTP Server . . . . .
POP3 Server . . . . .
Acoustic signal . . . . . off
Action . . . . . off
Cancel determination . . . . . on
Cancel determination and series . . . . . off

CALL **CALL coulometric KFT**

Call text	Track name	Condition	Condition
Start KFC	Coulometric KF titration	off	

REQUEST **REQUEST**

Sample data request
Sample position . . . . . off
ID1 . . . . . on
ID2 . . . . . on
ID3 . . . . . on
ID4 . . . . . off
ID5 . . . . . off
ID6 . . . . . off
ID7 . . . . . off
ID8 . . . . . off
ID9 . . . . . off
ID10 . . . . . off
ID11. . . . . off
ID12 . . . . . off
ID13 . . . . . off
ID14 . . . . . off
ID15 . . . . . off
ID16 . . . . . off
Sample size . . . . . on
Unit . . . . . off
Message . . . . . on

Add sample

TRACK **Coulometric KF titration**

Return immediately . . . . . on
Delete old data . . . . . off

KFC **KFC**

General/Hardware

Device
Device name . . . . . 831_1
Sensor
I(pol) . . . . . 10 µA
Electrode check . . . . . on
Cell
Generator type . . . . . without diaphragm
Generator current . . . . . 400 mA
Stirrer
Switch on/off automatically . . . . . on

Start conditions

Pause
Pause . . . . . 0 s

Control parameters

End point
EP at . . . . . 50.0 mV
Titration rate
Titration rate . . . . . optimal
Stop criterion
Stop criterion . . . . . rel.drift
Relative stop drift . . . . . 5 µg/min

Titration parameters

Extraction time . . . . . 300 s
Temperature . . . . . 25.0 °C
Time interval measuring point . . . . . 2 s

Stop conditions

Stop time . . . . . off s

Conditioning

Conditioning . . . . . on
Start drift . . . . . 20 µg/min
Drift correction . . . . . automatic
Stabilizing time . . . . . 0 s
Automatic start after sample addition time . . . . . off
Manual start after sample addition with [Continue] . . . . . on
Only start titration by a start command from a SEND command . . . . . off
Show measured value during conditioning . . . . . off

Additional evaluations

Fixed endpoint evaluation . . . . . off

Additional measured values

Additional calculated measured values . . . . . off
Additional external measured values . . . . . off

EXIT **Exit track**

CALC **CALC**

Result name	Formula	Unit	Decimal places	Assignment	Statistics
Water in Sample	= (('KFC.EP.QTY' - 'CV.Average KFC OVEN BLANK') / 'MV.Weight of Stopper Sample') * 100 / 1000	%	2	RS01	off
mg water per stopper	= ('KFC.EP.QTY' - 'CV.Average KFC OVEN BLANK') * 'MV.Weight of Entire Stopper' / 'MV.Weight of Stopper Sample' / 1000	mg water/stopper	2	RS02	off

Result name . . . . . **Water in Sample**
Formula . . . . . = (('KFC.EP.QTY' - 'CV.Average KFC OVEN BLANK') / 'MV.Weight of Stopper Sample') * 100 / 1000
Unit . . . . . %
Decimal places 2
Assignment RS01
Statistics . . . . . off
Description . . . . .
Result monitoring . . . . . off
Save result as common variable . . . . . off
Common variable . . . . .
Save result as titer . . . . . off
Solution name . . . . . 20 mL 807 buret

Result name . . . . . **mg water per stopper**
Formula . . . . . = ('KFC.EP.QTY' - 'CV.Average KFC OVEN BLANK') * 'MV.Weight of Entire Stopper' / 'MV.Weight of Stopper Sample' / 1000
Unit . . . . . mg water/stopper
Decimal places 2
Assignment RS02
Statistics . . . . . off
Description . . . . .
Result monitoring . . . . . off
Save result as common variable . . . . . off
Common variable . . . . . Average KFC OVEN BLANK
Save result as titer . . . . . off
Solution name . . . . . 0.1N Silver Nitrate

REPORT

REPORT Report template

Report template . . . . . Research Report
Report output
Printer . . . . . off
PDF file . . . . . on
PDF file . . . . . F:\SH\PD\EMPLOYEE FOLDERS\Voth, Laura\Tiamo pdfs\Reports.pdf
Send e-mail . . . . . off
Mail to . . . . .

DATABASE **DATABASE**

Database
Research Project Database

Karl Fischer Titration With THF Single Blank Method

Method parameters

Method . . . . . KFC 756 Sample Blank Research Project
Method saving date 2013-03-02 09:50:52 UTC-6
Method version 9
Method group . . . . . Research Methods
Method status . . . . . original
Method saved by (full name) . . . . . Laura Voth
Method saved by (short name) 10130396

START **Main track**

General

Workplace view
Current view . . . . . on
Track view for live window
Live display 1 . . . . . Coulometric KF titration
Live display 2 . . . . . Main track
Statistics . . . . . on
Number of single determinations 3
Conditioning
Automatic conditioning . . . . . on

Application note

Method variables

Name	Type	Assignment	Fixed value	Comment	Monitoring
Sample Name	Text	ID1		Sample identification 1	off
Manufacturer	Text	ID2		Sample identification 2	off
Lot #	Text	ID3		Sample identification 3	off
Expiry	Text	ID4			off

Name . . . . . **Sample Name**
Type . . . . . Text
Assignment . . . . . on ID1
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . . Sample identification 1

Name . . . . . **Manufacturer**
Type . . . . . Text
Assignment . . . . . on ID2
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . . Sample identification 2

Name . . . . . **Lot #**
Type . . . . . Text
Assignment . . . . . on ID3
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . . Sample identification 3

Name . . . . . **Expiry**
Type . . . . . Text
Assignment . . . . . on ID4
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . .

CALL **CALL coulometric KFT**

Call text	Track name	Condition	Condition
Start KFC	Coulometric KF titration	off	

REQUEST **REQUEST**

Sample data request
- Sample position . . . . . off
- ID1 . . . . . on
- ID2 . . . . . on
- ID3 . . . . . on
- ID4 . . . . . on
- ID5 . . . . . off
- ID6 . . . . . off
- ID7 . . . . . off
- ID8 . . . . . off
- ID9 . . . . . off
- ID10 . . . . . off
- ID11 . . . . . off
- ID12 . . . . . off
- ID13 . . . . . off
- ID14 . . . . . off
- ID15 . . . . . off
- ID16 . . . . . off
- Sample size . . . . . off
- Unit . . . . . off

Message . . . . . on

Add sample (blank) and enter required information

TRACK **Coulometric KF titration**

Return immediately . . . . . on

Delete old data . . . . . off

KFC **KFC**

General/Hardware

Device

Device name . . . . . 756_1

Sensor

I(pol) . . . . . 5 µA

Electrode check . . . . . on

Cell

Generator type . . . . . without diaphragm

Generator current . . . . . 400 mA

Stirrer

Switch on/off automatically . . . . . on

Start conditions

Pause

Pause . . . . . 0 s

Control parameters

End point

EP at . . . . . 50.0 mV

Titration rate

Titration rate . . . . . user

Control

Dynamics . . . . . 70.0 mV

Max. rate . . . . . 1800 µg/min

Min. rate . . . . . 15.0 µg/min

Stop criterion

Stop criterion . . . . . rel.drift

Relative stop drift . . . . . 11 µg/min

Titration parameters

Extraction time . . . . . 0 s

Temperature . . . . . 25.0 °C

Time interval measuring point . . . . . 2 s

Stop conditions

Stop time . . . . . off s

Conditioning

Conditioning . . . . . on

Start drift . . . . . 12 µg/min

Drift correction . . . . . automatic

Stabilizing time . . . . . 0 s

Automatic start after sample addition time . . . . . off

Manual start after sample addition with [Continue] . . . . . on

Only start titration by a start command from a SEND command . . . . . off

Show measured value during conditioning . . . . . off

Additional evaluations

Fixed endpoint evaluation . . . . . off

Additional measured values

Additional calculated measured values . . . . . off
Additional external measured values . . . . . off

EXIT **Exit track**

CALC **CALC**

Result name	Formula	Unit	Decimal places	Assignment	Statistics
Sample Blank	='KFC.EP.QTY'	µg/injection	2	RS01	on
Average KFC Sample Blank	= 'RS.Sample Blank.MNV'	µg/injection	2	RS02	off

Result name . . . . . **Sample Blank**
Formula . . . . . ='KFC.EP.QTY'
Unit . . . . . µg/injection
Decimal places 2
Assignment RS01
Statistics . . . . . on
Description . . . . .
Result monitoring . . . . . off
Save result as common variable . . . . . off
Common variable . . . . .
Save result as titer . . . . . off
Solution name . . . . . 20 mL 807 buret

Result name . . . . . **Average KFC Sample Blank**
Formula . . . . . = 'RS.Sample Blank.MNV'
Unit . . . . . µg/injection
Decimal places 2
Assignment RS02
Statistics . . . . . off
Description . . . . .
Result monitoring . . . . . off
Save result as common variable . . . . . on
Common variable . . . . . KFC Sample Blank
Save result as titer . . . . . off
Solution name . . . . . 0.1N Silver Nitrate

REPORT **REPORT**

Report template
Report template . . . . . Research Report
Report output
Printer . . . . . off
PDF file . . . . . on

PDF file . . . . . F:\SH\PD\EMPLOYEE FOLDERS\Voth, Laura\Tiamo pdfs\Reports.pdf
Send e-mail . . . . . off
Mail to . . . . . .

DATABASE **DATABASE**

Database

Research Project Database

Karl Fischer Titration With THF Three Blank Method - Blank #1

Method parameters

Method . . . . . KFC 756 Sample Blank #1
Research Project
Method saving date 2013-05-06 07:54:57 UTC-5
Method version 1
Method group . . . . . Research Methods
Method status . . . . . original
Method saved by (full name) . . . . . Dan Bowen
Method saved by (short name) 80014638

START **Main track**

General

Workplace view
Current view . . . . . on
Track view for live window
Live display 1 . . . . . Coulometric KF titration
Live display 2 . . . . . Main track
Statistics . . . . . on
Number of single determinations 3
Conditioning
Automatic conditioning . . . . . on

Application note

Method variables

Name	Type	Assignment	Fixed value	Comment	Monitoring
Sample Name	Text	ID1		Sample identification 1	off
Manufacturer	Text	ID2		Sample identification 2	off
Lot #	Text	ID3		Sample identification 3	off
Expiry	Text	ID4			off

Name . . . . . **Sample Name**
Type . . . . . Text
Assignment . . . . . on ID1
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . . Sample identification 1

Name . . . . . **Manufacturer**
Type . . . . . Text
Assignment . . . . . on ID2
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . . Sample identification 2

Name . . . . . **Lot #**
Type . . . . . Text
Assignment . . . . . on ID3
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . . Sample identification 3

Name . . . . . **Expiry**
Type . . . . . Text
Assignment . . . . . on ID4
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . .

CALL **CALL coulometric KFT**

Call text	Track name	Condition	Condition
Start KFC	Coulometric KF titration	off	

REQUEST **REQUEST**

Sample data request
- Sample position . . . . . off
- ID1 . . . . . on
- ID2 . . . . . on
- ID3 . . . . . on
- ID4 . . . . . on
- ID5 . . . . . off
- ID6 . . . . . off
- ID7 . . . . . off
- ID8 . . . . . off
- ID9 . . . . . off
- ID10 . . . . . off
- ID11 . . . . . off
- ID12 . . . . . off
- ID13 . . . . . off
- ID14 . . . . . off
- ID15 . . . . . off
- ID16 . . . . . off
- Sample size . . . . . off
- Unit . . . . . off

Message . . . . . on

Add sample (blank) and enter required information

TRACK **Coulometric KF titration**

- Return immediately . . . . . on

Delete old data . . . . . off

KFC **KFC**

General/Hardware

Device

Device name . . . . . 756_1

Sensor

I(pol) . . . . . 5 µA

Electrode check . . . . . on

Cell

Generator type . . . . . without diaphragm

Generator current . . . . . 400 mA

Stirrer

Switch on/off automatically . . . . . on

Start conditions

Pause

Pause . . . . . 0 s

Control parameters

End point

EP at . . . . . 50.0 mV

Titration rate

Titration rate . . . . . user

Control

Dynamics . . . . . 70.0 mV

Max. rate . . . . . 1800 µg/min

Min. rate . . . . . 15.0 µg/min

Stop criterion

Stop criterion . . . . . rel.drift

Relative stop drift . . . . . 11 µg/min

Titration parameters

Extraction time . . . . . 0 s

Temperature . . . . . 25.0 °C

Time interval measuring point . . . . . 2 s

Stop conditions

Stop time . . . . . off s

Conditioning

Conditioning . . . . . on

Start drift . . . . . 12 µg/min

Drift correction . . . . . automatic

Stabilizing time . . . . . 0 s

Automatic start after sample addition time . . . . . off

Manual start after sample addition with [Continue] . . . . . on

Only start titration by a start command from a SEND command . . . . . off

Show measured value during conditioning . . . . . off

Additional evaluations

Fixed endpoint evaluation . . . . . off

Additional measured values

Additional calculated measured values . . . . . off
Additional external measured values . . . . . off

EXIT **Exit track**

CALC **CALC**

Result name	Formula	Unit	Decimal places	Assignment	Statistics
Sample Blank	='KFC.EP.QTY'	µg/injection	2	RS01	on
Average KFC Sample Blank	= 'RS.Sample Blank.MNV'	µg/injection	2	RS02	off

Result name . . . . . **Sample Blank**
Formula . . . . . ='KFC.EP.QTY'
Unit . . . . . µg/injection
Decimal places 2
Assignment RS01
Statistics . . . . . on
Description . . . . .
Result monitoring . . . . . off
Save result as common variable . . . . . off
Common variable . . . . .
Save result as titer . . . . . off
Solution name . . . . . 20 mL 807 buret

Result name . . . . . **Average KFC Sample Blank**
Formula . . . . . = 'RS.Sample Blank.MNV'
Unit . . . . . µg/injection
Decimal places 2
Assignment RS02
Statistics . . . . . off
Description . . . . .
Result monitoring . . . . . off
Save result as common variable . . . . . on
Common variable . . . . . KFC Sample Blank #1
Save result as titer . . . . . off
Solution name . . . . . 0.1N Silver Nitrate

REPORT **REPORT**

Report template
Report template . . . . . Research Report
Report output
Printer . . . . . off
PDF file . . . . . on

PDF file . . . . . F:\SH\PD\EMPLOYEE FOLDERS\Voth, Laura\Tiamo pdfs\Reports.pdf
Send e-mail . . . . . off
Mail to . . . . . .

DATABASE **DATABASE**

Database

Research Project Database

Karl Fischer Titration With THF Three Blank Method – Blank #2

Method parameters

Method . . . . . KFC 756 Sample Blank #2 Research Project
Method saving date 2013-05-06 07:55:21 UTC-5
Method version 1
Method group . . . . . Research Methods
Method status . . . . . original
Method saved by (full name) . . . . . Dan Bowen
Method saved by (short name) 80014638

START **Main track**

General

Workplace view
Current view . . . . . on
Track view for live window
Live display 1 . . . . . Coulometric KF titration
Live display 2 . . . . . Main track
Statistics . . . . . on
Number of single determinations 3
Conditioning
Automatic conditioning . . . . . on

Application note

Method variables

Name	Type	Assignment	Fixed value	Comment	Monitoring
Sample Name	Text	ID1		Sample identification 1	off
Manufacturer	Text	ID2		Sample identification 2	off
Lot #	Text	ID3		Sample identification 3	off
Expiry	Text	ID4			off

Name . . . . . **Sample Name**
Type . . . . . Text
Assignment . . . . . on ID1
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . . Sample identification 1

Name . . . . . **Manufacturer**
Type . . . . . Text
Assignment . . . . . on ID2
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . . Sample identification 2

Name . . . . . **Lot #**
Type . . . . . Text
Assignment . . . . . on ID3
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . . Sample identification 3

Name . . . . . **Expiry**
Type . . . . . Text
Assignment . . . . . on ID4
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . .

CALL **CALL coulometric KFT**

Call text	Track name	Condition	Condition
Start KFC	Coulometric KF titration	off	

REQUEST **REQUEST**

Sample data request
- Sample position . . . . . off
- ID1 . . . . . on
- ID2 . . . . . on
- ID3 . . . . . on
- ID4 . . . . . on
- ID5 . . . . . off
- ID6 . . . . . off
- ID7 . . . . . off
- ID8 . . . . . off
- ID9 . . . . . off
- ID10 . . . . . off
- ID11 . . . . . off
- ID12 . . . . . off
- ID13 . . . . . off
- ID14 . . . . . off
- ID15 . . . . . off
- ID16 . . . . . off
- Sample size . . . . . off
- Unit . . . . . off

Message . . . . . on
Add sample (blank) and enter required information

TRACK **Coulometric KF titration**
- Return immediately . . . . . on

Delete old data . . . . . off

KFC **KFC**

General/Hardware

Device

Device name . . . . . 756_1

Sensor

I(pol) . . . . . 5 µA

Electrode check . . . . . on

Cell

Generator type . . . . . without diaphragm

Generator current . . . . . 400 mA

Stirrer

Switch on/off automatically . . . . . on

Start conditions

Pause

Pause . . . . . 0 s

Control parameters

End point

EP at . . . . . 50.0 mV

Titration rate

Titration rate . . . . . user

Control

Dynamics . . . . . 70.0 mV

Max. rate . . . . . 1800 µg/min

Min. rate . . . . . 15.0 µg/min

Stop criterion

Stop criterion . . . . . rel.drift

Relative stop drift . . . . . 11 µg/min

Titration parameters

Extraction time . . . . . 0 s

Temperature . . . . . 25.0 °C

Time interval measuring point . . . . . 2 s

Stop conditions

Stop time . . . . . off s

Conditioning

Conditioning . . . . . on

Start drift . . . . . 12 µg/min

Drift correction . . . . . automatic

Stabilizing time . . . . . 0 s

Automatic start after sample addition time . . . . . off

Manual start after sample addition with [Continue] . . . . . on

Only start titration by a start command from a SEND command . . . . . off

Show measured value during conditioning . . . . . off

Additional evaluations

Fixed endpoint evaluation . . . . . off

Additional measured values

Additional calculated measured values . . . . . off
Additional external measured values . . . . . off

EXIT **Exit track**

CALC **CALC**

Result name	Formula	Unit	Decimal places	Assignment	Statistics
Sample Blank	='KFC.EP.QTY'	µg/injection	2	RS01	on
Average KFC Sample Blank	= 'RS.Sample Blank.MNV'	µg/injection	2	RS02	off

Result name . . . . . **Sample Blank**
Formula . . . . . ='KFC.EP.QTY'
Unit . . . . . µg/injection
Decimal places 2
Assignment RS01
Statistics . . . . . on
Description . . . . .
Result monitoring . . . . . off
Save result as common variable . . . . . off
Common variable . . . . .
Save result as titer . . . . . off
Solution name . . . . . 20 mL 807 buret

Result name . . . . . **Average KFC Sample Blank**
Formula . . . . . = 'RS.Sample Blank.MNV'
Unit . . . . . µg/injection
Decimal places 2
Assignment RS02
Statistics . . . . . off
Description . . . . .
Result monitoring . . . . . off
Save result as common variable . . . . . on
Common variable . . . . . KFC Sample Blank #2
Save result as titer . . . . . off
Solution name . . . . . 0.1N Silver Nitrate

REPORT **REPORT**

Report template
Report template . . . . . Research Report
Report output
Printer . . . . . off
PDF file . . . . . on

PDF file . . . . . F:\SH\PD\EMPLOYEE FOLDERS\Voth, Laura\Tiamo pdfs\Reports.pdf
Send e-mail . . . . . off
Mail to . . . . . .

DATABASE **DATABASE**

Database

Research Project Database

Karl Fischer Titration With THF Three Blank Method – Blank #3

tiamo™ titration and more

License ID	3665614	Program version	tiamo 2.3 - 98 - 1
Computer name	MPWMJRGXH3		
User	10130396		2013-05-07 1:23:04 PM UTC-

Method parameters

Method . . . . . KFC 756 Sample Blank #3 Research Project
Method saving date 2013-05-07 13:22:45 UTC-5
Method version 2
Method group . . . . . Research Methods
Method status . . . . . original
Method saved by (full name) . . . . . Laura Voth
Method saved by (short name) 10130396

START **Main track**

General

Workplace view
Current view . . . . . on
Track view for live window
Live display 1 . . . . . Coulometric KF titration
Live display 2 . . . . . Main track
Statistics . . . . . on
Number of single determinations 3
Conditioning
Automatic conditioning . . . . . on

Application note

Method variables

Name	Type	Assignment	Fixed value	Comment	Monitoring
Sample Name	Text	ID1		Sample identification 1	off
Manufacturer	Text	ID2		Sample identification 2	off
Lot #	Text	ID3		Sample identification 3	off
Expiry	Text	ID4			off

Name . . . . . **Sample Name**
Type . . . . . Text
Assignment . . . . . on ID1
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . . Sample identification 1

Name . . . . . **Manufacturer**
Type . . . . . Text
Assignment . . . . . on ID2
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . . Sample identification 2

Name . . . . . **Lot #**
Type . . . . . Text
Assignment . . . . . on ID3
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . . Sample identification 3

Name . . . . . **Expiry**
Type . . . . . Text
Assignment . . . . . on ID4
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . .

CALL **CALL coulometric KFT**

Call text	Track name	Condition	Condition
Start KFC	Coulometric KF titration	off	

REQUEST **REQUEST**

Sample data request
- Sample position . . . . . off
- ID1 . . . . . on
- ID2 . . . . . on
- ID3 . . . . . on
- ID4 . . . . . on
- ID5 . . . . . off
- ID6 . . . . . off
- ID7 . . . . . off
- ID8 . . . . . off
- ID9 . . . . . off
- ID10 . . . . . off
- ID11 . . . . . off
- ID12 . . . . . off
- ID13 . . . . . off
- ID14 . . . . . off
- ID15 . . . . . off
- ID16 . . . . . off
- Sample size . . . . . off
- Unit . . . . . off

Message . . . . . on

Add sample (blank) and enter required information

TRACK **Coulometric KF titration**

- Return immediately . . . . . on

Delete old data . . . . . off

KFC **KFC**

General/Hardware

Device

Device name . . . . . 756_1

Sensor

I(pol) . . . . . 5 µA

Electrode check . . . . . on

Cell

Generator type . . . . . without diaphragm

Generator current . . . . . 400 mA

Stirrer

Switch on/off automatically . . . . . on

Start conditions

Pause

Pause . . . . . 0 s

Control parameters

End point

EP at . . . . . 50.0 mV

Titration rate

Titration rate . . . . . user

Control

Dynamics . . . . . 70.0 mV

Max. rate . . . . . 1800 µg/min

Min. rate . . . . . 15.0 µg/min

Stop criterion

Stop criterion . . . . . rel.drift

Relative stop drift . . . . . 11 µg/min

Titration parameters

Extraction time . . . . . 0 s

Temperature . . . . . 25.0 °C

Time interval measuring point . . . . . 2 s

Stop conditions

Stop time . . . . . off s

Conditioning

Conditioning . . . . . on

Start drift . . . . . 12 µg/min

Drift correction . . . . . automatic

Stabilizing time . . . . . 0 s

Automatic start after sample addition time . . . . . off

Manual start after sample addition with [Continue] . . . . . on

Only start titration by a start command from a SEND command . . . . . off

Show measured value during conditioning . . . . . off

Additional evaluations

Fixed endpoint evaluation . . . . . off

Additional measured values

Additional calculated measured values . . . . . off
Additional external measured values . . . . . off

EXIT **Exit track**

CALC **CALC**

Result name	Formula	Unit	Decimal places	Assignment	Statistics
Sample Blank	='KFC.EP.QTY'	µg/injection	2	RS01	on
Average KFC Sample Blank	= 'RS.Sample Blank.MNV'	µg/injection	2	RS02	off
Average KFC Sample Blank- 3 Bottles	= ('CV.KFC Sample Blank #1' + 'CV.KFC Sample Blank #2' + 'CV.KFC Sample Blank #3')/3		2	RS03	off

Result name . . . . . **Sample Blank**
Formula . . . . . ='KFC.EP.QTY'
Unit . . . . . µg/injection
Decimal places 2
Assignment RS01
Statistics . . . . . on
Description . . . . .
Result monitoring . . . . . off
Save result as common variable . . . . . off
Common variable . . . . .
Save result as titer . . . . . off
Solution name . . . . . 20 mL 807 buret

Result name . . . . . **Average KFC Sample Blank**
Formula . . . . . = 'RS.Sample Blank.MNV'
Unit . . . . . µg/injection
Decimal places 2
Assignment RS02
Statistics . . . . . off
Description . . . . .
Result monitoring . . . . . off
Save result as common variable . . . . . on
Common variable . . . . . KFC Sample Blank #3
Save result as titer . . . . . off
Solution name . . . . . 0.1N Silver Nitrate

Result name . . . . . **Average KFC Sample Blank- 3 Bottles**

Formula . . . . . = ('CV.KFC Sample Blank #1' + 'CV.KFC Sample Blank #2' + 'CV.KFC Sample Blank #3')/3
Unit . . . . . .
Decimal places 2
Assignment RS03
Statistics . . . . . off
Description . . . . . .
Result monitoring . . . . . off
Save result as common variable . . . . . on
Common variable . . . . . KFC Sample Blank
Save result as titer . . . . . off
Solution name . . . . . 0.1N Silver Nitrate

REPORT **REPORT**

Report template
Report template . . . . . Research Report
Report output
Printer . . . . . off
PDF file . . . . . on
PDF file . . . . . F:\SH\PD\EMPLOYEE FOLDERS\Voth, Laura\Tiamo pdfs\Reports.pdf
Send e-mail . . . . . off
Mail to . . . . . .

DATABASE **DATABASE**

Database

Research Project Database

Karl Fischer Titration With THF Stopper Sample Method

Method parameters

Method . . . . . KFC 756 Stopper by Number Research Project
Method saving date 2013-03-02 09:51:22 UTC-6
Method version 7
Method group . . . . . Research Methods
Method status . . . . . original
Method saved by (full name) . . . . . Laura Voth
Method saved by (short name) 10130396

START **Main track**

General

Workplace view
Current view . . . . . on
Track view for live window
Live display 1 . . . . . Coulometric KF titration
Live display 2 . . . . . Main track
Statistics . . . . . on
Number of single determinations 3
Conditioning
Automatic conditioning . . . . . on

Application note

Method variables

Name	Type	Assignment	Fixed value	Comment	Monitoring
Sample Name	Text	ID1		Sample identification 1	off
Lot #	Text	ID2			off
Volume of Sample Aliquot	Number	Sample size			off
Sample Size Unit	Text	Sample size unit			off
Number of Stoppers	Number	ID3			off
Total Volume of Extraction Solution	Number	ID4			off

Name . . . . . **Sample Name**
Type . . . . . Text
Assignment . . . . . on ID1
Fixed value . . . . . off . . . . .
Check at start . . . . . on
Comment . . . . . Sample identification 1

Name . . . . . **Lot #**
Type . . . . . Text
Assignment . . . . . on ID2

Fixed value . . . . . off
Check at start . . . . . on
Comment . . . . .

Name . . . . . **Sample Size Unit**
Type . . . . . Text
Assignment . . . . . on . . . . . Sample size unit
Fixed value . . . . . off
Check at start . . . . . on
Comment . . . . .

Name . . . . . **Volume of Sample Aliquot**
Type . . . . . Number
Assignment . . . . . on . . . . . Sample size
Fixed value . . . . . off
Check at start . . . . . on
Comment . . . . .
Variable monitoring . . . . . off
Lower limit . . . . .
Upper limit . . . . .
Message . . . . .
Display message . . . . . on
Record message . . . . . on
Message by e-mail . . . . . off
Use e-mail template . . . . . off
E-mail template . . . . .
Mail to . . . . .
Subject . . . . . Message from tiamo - Method 'KFC 756 Sample Blank Research Project' - Command 'Main track'
User . . . . .
Mail from . . . . .
SMTP Server . . . . .
POP3 Server . . . . .
Acoustic signal . . . . . off
Action . . . . . off
Cancel determination . . . . . on
Cancel determination and series . . . . . off

Name . . . . . **Number of Stoppers**
Type . . . . . Number
Assignment . . . . . on ID3
Fixed value . . . . . off
Check at start . . . . . on
Comment . . . . .
Variable monitoring . . . . . off
Lower limit . . . . .

Upper limit
Message
Display message . . . . . on
Record message . . . . . on
Message by e-mail . . . . . off
Use e-mail template . . . . . off
E-mail template
Mail to
Subject . . . . . Message from tiamo - Method 'KFC 756 Sample Blank Research Project' - Command 'Main track'
User
Mail from
SMTP Server
POP3 Server
Acoustic signal . . . . . off
Action . . . . . off
Cancel determination . . . . . on
Cancel determination and series . . . . . off

Name . . . . . **Total Volume of Extraction Solution**
Type . . . . . Number
Assignment . . . . . on ID4
Fixed value . . . . . off
Check at start . . . . . on
Comment
Variable monitoring . . . . . off
Lower limit
Upper limit
Message
Display message . . . . . on
Record message . . . . . on
Message by e-mail . . . . . off
Use e-mail template . . . . . off
E-mail template
Mail to
Subject . . . . . Message from tiamo - Method 'KFC 756 Sample Blank Research Project' - Command 'Main track'
User
Mail from
SMTP Server
POP3 Server
Acoustic signal . . . . . off
Action . . . . . off
Cancel determination . . . . . on
Cancel determination and series . . . . . off

CALL **CALL coulometric KFT**

Call text	Track name	Condition	Condition
Start KFC	Coulometric KF titration	off	

REQUEST **REQUEST**

Sample data request
- Sample position . . . . . off
- ID1 . . . . . on
- ID2 . . . . . on
- ID3 . . . . . on
- ID4 . . . . . on
- ID5 . . . . . off
- ID6 . . . . . off
- ID7 . . . . . off
- ID8 . . . . . off
- ID9 . . . . . off
- ID10 . . . . . off
- ID11 . . . . . off
- ID12 . . . . . off
- ID13 . . . . . off
- ID14 . . . . . off
- ID15 . . . . . off
- ID16 . . . . . off
- Sample size . . . . . on
- Unit . . . . . on

Message . . . . . on
- Add sample and enter required information

TRACK **Coulometric KF titration**
- Return immediately . . . . . on
- Delete old data . . . . . off

KFC **KFC**

General/Hardware
- Device
  - Device name . . . . . 756_1
- Sensor
  - I(pol) . . . . . 5 µA
  - Electrode check . . . . . on
- Cell
  - Generator type . . . . . without diaphragm
  - Generator current . . . . . 400 mA
- Stirrer
  - Switch on/off automatically . . . . . on

Start conditions
Pause
Pause . . . . . 0 s
Control parameters
End point
EP at . . . . . 50.0 mV
Titration rate
Titration rate . . . . . user
Control
Dynamics . . . . . 70.0 mV
Max. rate . . . . . 1800 µg/min
Min. rate . . . . . 15.0 µg/min
Stop criterion
Stop criterion . . . . . rel.drift
Relative stop drift . . . . . 11 µg/min
Titration parameters
Extraction time . . . . . 0 s
Temperature . . . . . 25.0 °C
Time interval measuring point . . . . . 2 s
Stop conditions
Stop time . . . . . off s
Conditioning
Conditioning . . . . . on
Start drift . . . . . 12 µg/min
Drift correction . . . . . automatic
Stabilizing time . . . . . 0 s
Automatic start after sample addition time . . . . . off
Manual start after sample addition with [Continue] . . . . . on
Only start titration by a start command from a SEND command . . . . . off
Show measured value during conditioning . . . . . off
Additional evaluations
Fixed endpoint evaluation . . . . . off
Additional measured values
Additional calculated measured values . . . . . off
Additional external measured values . . . . . off

EXIT **Exit track**

CALC **CALC**

Result name	Formula	Unit	Decimal places	Assignment	Statistics
Water in Sample	='KFC.EP.QTY'	µg	2	RS01	on

Result name	Formula	Unit	Decimal places	Assignment	Statistics
Water per Stopper	= ('RS.Water in Sample.MNV' - 'CV.KFC Sample Blank') * ('MV.Total Volume of Extraction Solution') * 1000 / 'MV.Volume of Sample Aliquot' / 'MV.Number of Stoppers' / 1000	mg	2	RS02	off

Result name . . . . . **Water in Sample**
Formula . . . . . ='KFC.EP.QTY'
Unit . . . . . µg
Decimal places 2
Assignment RS01
Statistics . . . . . on
Description . . . . .
Result monitoring . . . . . off
Save result as common variable . . . . . off
Common variable . . . . .
Save result as titer . . . . . off
Solution name . . . . . 20 mL 807 buret

Result name . . . . . **Water per Stopper**
Formula . . . . . = ('RS.Water in Sample.MNV' - 'CV.KFC Sample Blank') * ('MV.Total Volume of Extraction Solution') * 1000 / 'MV.Volume of Sample Aliquot' / 'MV.Number of Stoppers' / 1000
Unit . . . . . mg
Decimal places 2
Assignment RS02
Statistics . . . . . off
Description . . . . .
Result monitoring . . . . . off
Save result as common variable . . . . . off
Common variable . . . . . KFC Sample Blank
Save result as titer . . . . . off
Solution name . . . . . 0.1N Silver Nitrate

REPORT **REPORT**

Report template
Report template . . . . . Research Report
Report output
Printer . . . . . off
PDF file . . . . . on
PDF file . . . . . F:\SH\PD\EMPLOYEE FOLDERS\Voth, Laura\Tiamo pdfs\Reports.pdf
Send e-mail . . . . . off

DATABASE **DATABASE**

Database
Research Project Database